

nature*August 5, 1976*

Still a lot to learn from the Chinese

TEN thousand, a hundred thousand, a million dead in the latest earthquake at Tangshan in China? It seems as if the Chinese authorities are not at present going to talk about the scale of the disaster, maybe believing that details of fatalities will produce some sort of ghoulish exultation in other countries and result in an indefinable loss of face. Certainly some silly people who, a year ago, would have dubbed the prediction which saved tens of thousands of lives on the Liaoning Peninsula a "spectacular success" have been talking about the present catastrophe as a "spectacular failure" of prediction. Such views can no doubt be discounted as shallow, but they do perhaps show why China will be sensitive to worldwide comment, and they do make it all the more necessary that the Chinese should understand the serious and compelling reasons why earthquake scientists, engineers and social scientists the world over want to learn as many lessons as quickly as possible from China, now an acknowledged leader in the field of prediction.

Even though there do not seem to have been any open alerts about the most recent quake, those who have recently returned from China report that there had been concern over magnetic field changes and that there was a feeling that seismic activity was migrating in the general direction of Tangshan. More remarkably, travellers continue to bring back accounts of unusual phenomena preceding and accompanying earthquakes, which seem to indicate that there is still a lot to learn about the art of prediction.

Animal behaviour as a predictor has now, of course, become an object of popular fascination the world over. For one thing, the jumpiness of cats, dogs and snakes can be understood by a much wider public than can fluctuations in the velocity of elastic waves; for another, it does bring science down to a folksy level at which the good amateur observer is just as valued as the professional. There are few branches of science where this is true today, and it is excellent news that amateur involvement has been given such a good name by the Chinese. Even the most sceptical Western observer will be im-

pressed by the sheer crescendo of reportings of unusual phenomena last year in the weeks before the Liaoning earthquake. The latest addition to the collection is 'earthquake lights'. Like anomalous animal behaviour, this has been a subject attracting practically no attention at all from seismologists, yet there is now a report from a respected Chinese seismologist of a glow in the air at the same time as a quake—something that had previously only been the subject of discounted hearsay.

What the Chinese have succeeded in doing, and what this most recent calamity will certainly not dissuade them from continuing, is to raise the consciousness of people to unusual phenomena, even to the extent of encouraging them to file reports. This is a lesson that should not be lost in California, the Soviet Union, Japan, the Middle East or other places scourged by earthquakes. There are encouraging signs that at least the inhabitants of San Francisco and Los Angeles are prepared to learn from the Chinese experience that the experts need all the help they can get. That is why it is important that the momentum of interest which has been developing should not be stifled by suppression of news and of the interchange of scientists.

There is a lesson in all this for the international organisations, too. Countries which have developed technologies for their home market invariably start to turn their thoughts to export potential before long. And if the technology is one like railways, air-traffic control or defence systems, the inevitable interchange of personnel, training of technicians and stationing of advisers creates a dependency which is a modern form of imperialism. Just because China is at present reeling under a disaster and no other country can yet do any better does not mean that in ten years time there will not be an exportable commodity—and that certain countries, already with tribulations enough of their own, will not be asked to buy an expensive system and absorb a thousand advisers. UNESCO, rather well equipped in this field, should take this matter seriously while it is still a cloud on the horizon. □



The ecology of ecology

"Ecology has become a social movement as well as a branch of biology". Philip Lowe of University College, London, and Michael Worboys of Sheffield City Polytechnic give their view on the role of science in the environmental movement

ENVIRONMENTAL concern, and with it the apparent immediacy of the ecological crisis, has of late receded. A downturn in the business cycle has set its own limits to growth, and apocryphal warnings of the decline of Western capitalist democracies have superseded the environmentalists' warnings of natural decay. But many environmental problems remain, and one enduring feature of many of these is their implication of modern science, both as cause and as solution.

From the anxiety over radioactive fall-out and pesticides, through to current worries about fluorocarbons, nuclear power and hazards of the workplace, science has often been cast as the villain of the piece. Yet scientists have also been in the forefront in alerting society about these problems and in campaigning for action. Moreover, a leading role for science has been proposed in evaluating risks from pollution, in developing new resources and in the general formulation of solutions and strategies in response to environmental problems.

This ambivalence with regard to science—which is, incidentally, very common where science impinges upon social issues—creates many difficulties. For example, different scientists may regard the same "problem" very differently. Whose assessment or warning do we believe? To solve environmental problems, do we need more science, less science or different science? To which group of scientists, if any, do we turn for solutions? One common response to questions of this sort has been to look to ecology and ecologists as sources of "wisdom" and "healing". The received notion that ecology is about wholeness and the natural has left it seemingly untainted by the reductionist and industrial associations of most modern science.

Indeed, its marginality to mainstream science, of which ecologists have long complained, now proves to be an asset

in a period when science generally is being questioned and attacked. Most environmentalists and environmental pressure groups have fallen in behind the banner of Ecology. They have used ecological information vicariously and promoted the adoption of ecological values, giving rise to a whole new genre of social polemic best described as popular ecology. In short, ecology has become a social movement as well as a branch of biology, and it is the connection between these two aspects of ecology that needs to be explored.

Is politics redundant?

The literature of the contemporary environmental movement and popular ecology is characterised by a general rejection of conflict, especially political conflict: the lesson of ecology in its broadest sense is supposedly harmony, balance and symbiosis. As in technocracy, where traditional politics is deemed redundant through the overriding and deterministic effects of man's technological situation, so in popular ecology politics is declared equally redundant because of the imperatives of man's ecological predicament. Compelling physical and natural constraints supersede and replace moral, social and political considerations.

In Britain, the establishment of an Ecology Party indicates the presumption that environmental issues transcend orthodox political alignments. Formed in 1973 as a direct consequence of the appeal in the *Blueprint for Survival* for "a national movement to act at national level, and if need be to assume political status and contest the next general election", it was known initially as the People Party—its subsequent change of name illustrating nicely how ecology has come to be seen as bigger than all of us. The electoral ambitions of the Ecology Party, its commitment to come to power by democratic means and work within the framework of a mixed capitalist economy and a parliamentary

democracy, are somewhat at odds with its very radical manifesto proposals for de-growth, decentralisation and population control "to provide a working basis for ecological government". However, recent correspondence in their broadsheet, *Alliance*, has argued that it may in fact be essential for government, composed of an aware elite—the writer used the Spaceship Captain analogy—"to introduce ecology without taking the people into its confidence".

But most environmentalists would not completely abolish politics. Some view politics as the refined tool of environmental management, enabling fine and delicate control of human well-being within the greater and larger purposes of the biosphere. The implication is that, as well as transcending political differences, environmental problems are also non-political in the sense that their solutions are management or technical issues. Hence, politics should no longer be about power, its use and distribution, but, instead, about the forms or techniques of government most appropriate to cope with urgent environmental problems.

Conventional response

The conventional response of the scientific establishment to environmental problems was expressed by the Council for Scientific Policy in 1972, when it urged that "more science will be needed, not less, to provide remedies and to enable society to take precautions against pollution", and called for the "closer integration of science into social policies". Thus, more "scientific" planning, monitoring and management are advocated, in such forms as technology assessment, environmental impact analysis, quantitative environmental forecasting and ecological evaluation—activities viewed by their practitioners as so many exercises in applied rationality, but criticised by others for clothing implicit political assumptions and implications in a pseudo-scientific mystique of "objective" knowledge and "rational" techniques. What is clear is that their practice seems to be based on the assumption of the availability of consensually defined social goals which, if absent, can be summonsed by the persuasive presentation of the facts of the matter.

Ecological coalition

Conservation, when treated in these terms, appears to be an unadulterated good thing; equated with applied ecology and with its own quantitative techniques, it seems beyond the realm of politics. The supposed political consensus surrounding the practice of conservation in Britain, however, can also be seen to be the operation of a coalition of powerful rural vested interests, whose use of ecological rhetoric has enjoyed the active support or acquiescence of professional ecologists. In fact, popular ecology has long been very closely linked with the interests and ambitions of professional ecologists.

The institutionalisation of a scientific discipline such as ecology is generally accompanied by the competitive development of a disciplinary ideology which publicises the putative social utility and world-view of the discipline and plays an essential role in the establishment of cohesion and a sense of collective identity amongst its practitioners. In Britain, the emergence of ecology as a distinct discipline dates from the turn of the century. Government recognition of its potential utility came in 1949 with the establishment of the Nature Conservancy as both a research council for ecology and an agency for the formation and management of nature reserves.

Initially, the Nature Conservancy commanded a very much smaller share of resources than the other research councils, and ecology, with its amateur connotations of natural history, had a low status in the scientific community. But the Conservancy proved very successful in lobbying government and popularising ecology, and took the leading role in forging various groups into an effective and dynamic environmental lobby. In the late 1960s members of the Conservancy played a crucial part in popularising the existence of an environmental crisis and figured prominently in the institutional developments that were the outcome of the resulting public and governmental concern. Hence a strong element in ecological propaganda is a direct product of competition within science.

Whose preserve?

The further suggestion that the environmental crisis reflected the pre-eminence of the physical sciences over the environmental sciences has also had considerable currency. It is indeed true that many science-related activities associated with both the generation and control of environmental problems, have been the preserve of chemists and engineers, largely to the exclusion of biologists. Thus, much pollution, such as that due to pesticides, detergents

and nuclear radiation, has been seen to be consequent upon faulty and diminutive knowledge of environmental biology as compared with, say, physics and chemistry—indicating to some the need for a greater share of resources to be devoted to research and training in the environmental sciences.

To many others, however, this analysis, in confining itself to relative deficiencies within science, does not go far enough and fails to recognise the economic and social basis of the organisation of science. The preponderance of the chemical control of pests over their biological control, for example, and the resultant pollution and development of pest resistance, may be less a function of the power of chemists over biologists than of the power of the chemical industry within society. Professional competition and interfactional disputes within science often need to be understood in terms of the relationship between scientific disciplines and elites and powerful interests external to science.

Beyond tactical responses

The plausibility of ecologists has been of crucial importance, and was directly involved in their popularisation of the environmental crisis, just as their professional interests are deeply enmeshed in "technical" solutions to environmental problems. In some instances, however, the appeal to ecology has gone beyond the search for tactical responses to particular problems, towards the claim that ecology can contribute to a radical re-ordering of human purposes and a re-orienting of society away from environmental disaster. The findings, concepts and approach of ecology have proved a rich source of ideas and analogies in the construction of environmentally sound social programmes—niche theory, the ecosystem concept, energy flow, materials recycling, limits of tolerance and succession have been deployed in many and varied contexts.

Beyond this have come calls for ecological ideals, an ecological ethic, an ecological morality, ecological values and ecological determinism. Certainly, the synthetic nature of ecology and its claims to transcend the narrowness of other disciplines have always been a source of its popular support. But, in this case, science appears to be adopting a leading role in society as a value-affirming, goal-setting and consensus-generating institution. At a time of change and dissent from established values, when it is generally accepted that traditional institutions have lost much of their authority, science, albeit reunified and chastened by the example of ecology, is turned to by those who value order and stability as the one authoritative voice.

Science assailed

Such appeals to science, however, come at a time when its own moral influence has also been assailed, by the attacks of what many identify as an anti-science movement. The response of critics like Roszak to the social problems of modern science has been to reject science—the objective, rational and calculating consciousness—in favour of a more subjective, intuitive and feeling type of knowledge. For Roszak, the discipline of ecology, and not high energy physics, should be the model science—a view shared by influential and respected proponents of ecology such as Max Nicholson and Barry Commoner.

The separate debate over social responsibility in science has raised other questions about the direction and control of science. The self-doubt that has been generated has prompted some leading scientists to demand greater solidarity and less dissent within the scientific community so as to render it less vulnerable to external attack and to preserve its authority in society. Indeed, internal fragmentation has been identified as one cause of the inability of science to respond effectively to environmental problems, and calls for a "unified science", based on ecology and the related general systems theory, predict the consequent restoration of the weakened position of science in society. The holistic image of ecology, its promise of making whole that which was fragmented, and its widespread popular support, might help revive the moral influence of science, and regenerate consensus both within and outside of science.

Crisis highlighted

The environmental crisis thus highlights a crisis of confidence within science and a crisis of science's authority in society. Mary Douglas, in her essay "Environments at Risk" (*Times Literary Supplement*, October 30, 1970), has supported this view by drawing attention to the problems of credibility that scientists face in warning society of possible environmental dangers. Indeed, the environmental movement has been interpreted by Michael King (*Technology and Society*, 8, 1; 1973) as a bid by certain scientists to relocate and extend the basis of scientific authority. Science as a mere source of useful knowledge confers upon scientists the very limited and fragile power of technicians, but the ecologists' claim to appreciate the wisdom of Nature, as well as the orthodox scientists' knowledge of natural laws, might legitimate a continuous and central involvement in the formulation of political purposes and values. □

Science policy in Poland

Science has not escaped recent developments in Poland. Below, a Special Correspondent describes the trends.

AFTER four relatively fat years science in Poland seems to be entering a less happy period. Following the dismissal of Mr Gomulka during the stormy days of December 1969, his successors initiated a more dynamic economic policy and science was declared an essential factor of economic growth. The new leaders were keen to present themselves as scientifically oriented people. A honeymoon for science began with the setting up of a special committee, headed by the well-known sociologist Professor Jan Szczepanski, to develop long term policy in education at all levels. The state pocket was enriched by loans from abroad and was opened wider for science.

As a result of this, research and development expenditure rose from 1.4–1.6% of GNP in 1969 to about 2.5% in 1975. GNP itself rose by about 60% during this period and so the funds available for science were almost doubled—a dramatic increase, especially in comparison with stagnating funds for science in Western countries. These official figures perhaps exaggerate the increase to some extent because the definition of what is meant by science for budgeting purposes is vague and some expenditures not included previously are now under the heading of science. A sharp rise in the money available is, however, an indisputable feature of this period and witnessed by a number of new buildings, better endowment of institutes and laboratories, improvement of the level of scientific work in many fields and wider contacts between Polish and foreign scientists.

The rise of money was not accompanied by a proportional rise of scientific staff or number of students. In the 1950s the relative number of students in Poland was greater than in most Western countries. But since the further rise of the number of students was slow (especially after the student riots in 1968) Poland has been overtaken by most European countries. The accompanying table of numbers from the official Statistical Yearbook, 1975, shows the recent trends.

It has never been easy in Poland to spend all the money allocated for science, and it may be even more difficult in the future. Budgets of Universities and Institutes are being tightened by a large number of regulations more relevant to industry than to science. This is a feature of the five-year plan for the 1976–1980 period for the development of different aspects of technology. Plans of this kind—strange

as it sounds—have also been compiled for basic science. A low opinion of the value of the whole time-consuming and inelastic planning system is common in the scientific community.

In spite of the earlier generous flow of money the housing conditions of many scientific institutions remain poor. Many courses in the University of Warsaw are given in buildings belonging to different secondary schools dispersed over the city of Warsaw. The salaries of academic staff are very small in relation both to academic salaries in other countries of the communist block and salaries of other professional groups in Poland. The salary of a senior member of the staff (*adiunkt*) of a research institute is equal to the average salary of those working in the state sector (about 3500 zlotys per month, that is, \$120 on the official tourist exchange rate—a figure which gives an accurate impression of the buying power of the earnings). From September 1976 the salaries will rise by about 15%. The rise of salaries previously given to all other professional groups is now reaching the last group of government employees, namely, those working in the scientific institutes.

The honeymoon of the Polish scientific intelligentsia was over in 1973, close to the time of the much advertised Second Congress of Polish Sciences. It seems that the reason for this change of the official attitude was related to the disillusion of the government that Polish science will contribute significantly to the immediate improvement of the economy. Unfortunately these idle hopes have been repeatedly supported by a large number of short-sighted Polish scientists eager to satisfy their personal goals.

In Poland as in most eastern-block countries the intermediate link between scientific research and technology, the link responsible for implementation of new products and processes, is very weak. Its functions are frequently performed with doubtful results by purely

academic institutions, for example in the institutions of the Polish Academy of Sciences (PAS). Thus the sharp rise of the level of Polish industry in 1970–75 was only marginally based on the achievements of Polish scientists and engineers, and was due mainly to the purchase of know-how from abroad; noting this, the leaders of the country have begun to treat science not as a producer of goods but as a consumer.

The social sciences in Poland depend on official doctrine to a lesser extent than in other countries of the communist block. However, in spite of the official statements made in 1970 interest in their work has declined steadily. The report of the education committee under Szczepanski has been shelved, and the education policy actually introduced bears little relation to its conclusions. Poland was fortunate to have after the war three economists of international distinction, Oscar Lange, Michael Kalecki and Edward Lipinski, but their economic advice has been used in several other countries and never in Poland. Of these three only Professor Lipinski, member of PAS, is alive, but his views expressed in a number of letters having a wide hand-to-hand circulation are not taken into account by the authorities.

In contrast to other groups of Polish intellectuals the scientists are relatively meek. Not many scientists or scholars signed the letters of protest against the recent revision of the Polish constitution legalizing the leading role of the party and friendship to the USSR. Those who did are being punished: younger people are not having their contracts renewed, and others, as for example Professor Stefan Amsterdamski, philosopher, are prohibited from going abroad even to attend conferences.

According to official statements the government is not planning to increase the quota of places for students in universities. This means that the situation of an applicant will be about the same as during the last few years due to the fact that the post-war demographical bulge is now close to its end. The announced quota based on the evaluation of future demands is very conservative and recently it became clear that for example the number of

Student trends in Poland

Year	No. of students per 10,000 of population	No. of resident students per 10,000 of population	No. of students per 1,000 of population in the age group 19–24	No. of resident students per 1,000 of population in the age group 19–24
1970–71	101	64	136	87
1974–75	126	76	139	84

The official number of students per 10,000 of population given in the international part of the Yearbook is 144, but this includes also students in higher schools under the Ministry of Defence and the Ministry of Internal Affairs. In these schools there are about 80,000 students, and the total number of students in other institutions of higher education is 427,000.

physicians needed exceeds the number of accepted medical students.

During the academic year 1975-76 there was a considerable rise of political activity among students. They raised their voices in protest against the changes in the constitution. More recently they protested against the expulsion of their colleague Mr Jacek Smykala from the Medical Academy in Szczecin (during a tutorial class in economics he asked what part of the national budget is allocated to the police force), and against the imprisonment of their colleague Mr Stanislaw Kruszynski from the Catholic University in Lublin (the only private university in Poland), who was sentenced to six months in prison for writing unfavourable opinions about the social system in Poland in private letters to his relatives and friends. From Warsaw University alone about 800 people signed this protest.

At the beginning of 1976 a reduction of staff (up to 10%) took place in many research institutes, including those connected directly with industry. These reductions touched universities and industries of PAS to a lesser extent but it was decided to freeze the number of their staff for the next three years.

The Polish economy, as is commonly accepted, is now entering a very difficult period. One of the signs of this was the government's proposals to increase the food prices in June (meat by about 70%, sugar 100% and so on) which would have caused a fall in the spend-

ing power of wages by about 20 to 30%. The wave of strikes (70 to 80% of big factories according to unofficial figures) and unrest in a few places forced the government to rescind this proposal. Science seems to be near the top of the list for economies. According to some sources it has already been decided to halve the rate of investment in science. Considerable political tension in the whole country has not by-passed the scientists. Speculations about personal changes have wide circulation. Meanwhile the repressions against those involved in strikes and protests have already begun.

As in all countries of the communist block, scientists in Poland have no real representation to defend their own interests and those of science. The Polish Academy of Sciences is the agent of the government and provides no more than formal representation. For example, recently, in June 1976, after the abortive attempt to increase prices, the Praesidium of PAS at its meeting in the metallurgical factory in Silesia wrote a letter of adulation to Mr Gierek, the Secretary of the Party. A few months previously, his son, Professor Adam Gierek, was elected to the PAS; perhaps those who voted in his favour had in mind the dire fate of the Romanian Academy, which, as it is said, was a consequence of its refusal to elect Mrs Ceausescu (wife of the Secretary of the Party in Romania) to the Academy.

These and similar steps do not con-

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Polish leader Edward Gierek

tribute to the authority of PAS and do not reflect the views of the Polish scientific community. An interesting new development in this community and in the whole group of intellectuals is the collaboration of differently minded people, rightists and leftists, catholics and even members of the party. This collaboration is an outcome of the common feeling that the present policy endangers not only the further development of science, but also the very existence of independent Poland, and that the most important task confronting the intelligentsia is the defence of the national identity. □

Artificial intelligence in education

Miranda Robertson discusses research aimed at exploiting computers in human communication and teaching

IF the principal goal of research in artificial intelligence (AI) has been the development of intelligent machines, an increasingly conspicuous sideline is the promotion of intelligence in human beings. This social orientation has been stimulated partly by Newell and Simon at Carnegie Mellon, and the ideas of Papert and Minsky, at the Massachusetts Institute of Technology, (MIT), who see in artificial intelligence a new way of looking at the nature and development of natural intelligence.

Papert has for some years been an advocate of the use of sophisticated computer programming languages to help children learn how to think. Writing a program in a high-level language involves the expression of thought processes which would not usually be formulated. When there is a mistake ("bug") in the program, the child can retrace his thoughts in

the program and understand his own mistake. The result, Papert believes, is to increase the child's intellectual effectiveness in general and make him more articulate.

LOGO learning for children

That is the idea behind experiments which have been going on in the LOGO laboratory at MIT for about three years. LOGO is a high level language which children can use to program various devices, the best known of which is a mechanical "turtle" with a retractable pen.

Usually, the child begins by learning how to draw geometric designs by programming the turtle; later, as the system becomes more familiar, the turtle can be replaced by a somewhat nimbler oscilloscope trace. One important advantage of a LOGO system over more conventional curricula is that children enjoy working with it

and it can be used to overcome the resistance that many children develop to learning mathematics.

So far, the experiments at MIT have not been sufficiently systematic to produce clear evidence for Papert's persuasive arguments. Up to now, the children have been drawn largely from the families of MIT research workers and from a local school, neither (probably) very representative; and the classes have been supervised by research staff, in particular Dr Jeanne Bamberger, rather than by professional teachers.

There has for some time been talk of an entire school geared to the LOGO system, and where about half the school curriculum would be undertaken at a computer terminal. But this ambitious project is not expected to start in earnest for another two years, during which a small group of teachers will be trained in LOGO, a pilot LOGO educational project will be run with the cooperation of one of the Cambridge or Boston schools, and some thought will be given to how the children's intellectual development should be evaluated.

Evaluation presents a special problem because it is not clear that the intellectual abilities LOGO is supposed to foster would necessarily be revealed by conventional psychometric tests. Papert and his colleague Marvin Minsky, who specialises in theories of intelligence, maintain not only that conventional tests are inappropriate, but that the qualitative difference they would expect to see in the children would be great enough to make statistical tests unnecessary. On the other hand they do not expect to be able to show those differences with anything short of a total "LOGO environment" and that is two or three years off.

In the meantime, however, their ideas have inspired a number of schools and universities elsewhere to set up their own experiments with LOGO or other similar systems. Of those, a small research project and the Artificial Intelligence Department at the University of Edinburgh is the

furthest advanced with a scheme for providing more rigorous experimental evidence. Jim Howe, Tim O'Shea and Ben du Boulay have enlisted the cooperation both of a local school and of a teacher training college with the aim of finding out how learning may not only help pupils to learn, but teachers to teach. Eleven-year-old children from George Herriots, a direct grant Edinburgh boys' school, have now been taking part for a year in LOGO classes in the course of which a LOGO curriculum has been designed, and practical experience has led to various improvements both in the language itself and to the development of new devices.

Children with problems

Although the Edinburgh group do not entirely share Minsky's and Papert's contempt for all psychological testing (indeed part of the research project is to design suitable tests), the

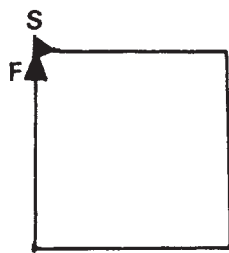
most striking effects they have seen so far have been precisely of a kind that psychometric tests would miss. Some of the most interesting evidence so far is anecdotal, and the example of one particular child with a problem suggests that a particular advantage of LOGO learning may be in bridging the communications gap between teacher and pupil, especially where for one reason or another that is particularly difficult.

The anecdotal evidence concerns a "dyslexic" boy who was included by chance in the first group of 18 children to be taught LOGO. In spite of remedial lessons from sympathetic and enthusiastic specialists for two years, neither his reading and writing nor his classroom performance had improved, and he was well established in the role of class clown and dunce. LOGO programming, unlike the rest of his school work, however, came relatively easily to him and he rapidly reached a point where he was able to teach other boys. That brought about a change in his classmates' attitude to him and a conspicuous increase in his own self-confidence. O'Shea illustrates the point with an account of an argument between the boy and his teacher during a classroom lesson on sets. Asked to name the set of even numbers divisible by two and the set of odd numbers divisible by two, he responded with, "the set that never finishes and the set that never starts". The textbook answers are the infinite set and the empty set, and the teacher marked him wrong.

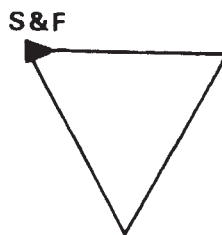
In a way that would have been uncharacteristic before he started learning LOGO, the boy argued with his teacher and as a result was awarded half marks. Part of the significance of that story, for Howe and O'Shea, lies in the phrasing of the boy's "wrong" response, which, like the definition of a LOGO program, is expressed in terms of procedures. The implication is that learning LOGO supplied not only the confidence to argue, but the language in which to do so (incidentally, a school inspector spontaneously remarked of other boys in the group that they were exceptionally mathematically argumentative).

LOGO learning for teachers

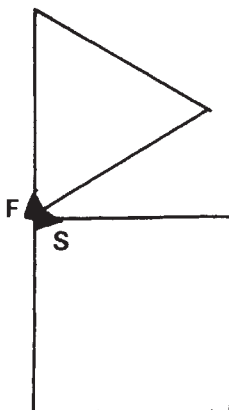
One of Papert's most important claims has been that learning LOGO provides a descriptive system within which to think and communicate; and this is relevant not only to the private intellectual development of individual children, but to the interaction between pupil and teacher: it can also help the teacher to understand and express what he has to teach. Papert maintains that what makes so many common skills (such as riding a bicycle) so hard



w: TO SQUARE
d:1 FORWARD 100
d:2 RIGHT 90
d:3 FORWARD 100
d:4 RIGHT 90
d:5 FORWARD 100
d:6 RIGHT 90
d:7 FORWARD 100
square defined
w: SQUARE



w: TO TRIANGLE
d:1 FORWARD 100
d:2 RIGHT 120
d:3 FORWARD 100
d:4 RIGHT 120
d:5 FORWARD 100
d:6 RIGHT 120
d: END
triangle defined
w: TRIANGLE



w: TO HOUSE
d:1 SQUARE
d:2 TRIANGLE
END
house defined
w: HOUSE

This is an example of a "bug" which is commonly encountered when, having learned to write procedures to draw a square and a triangle, the child puts the two programs together to make a "house". In this particular case, the bug arises because the turtle's change in orientation (indicated by arrows: S=starting position, F=finishing position) in the course of drawing the two shapes has not been correctly predicted.

w: prompt typed when the computer is ready to take a command; d: prompt typed when computer is in the definition mode.

to teach is the lack of an adequate way of describing them. Because learning with LOGO is learning within a descriptive system, it makes everything explicit and thus automatically more communicable.

That is the rationale for an experimental LOGO course which Ben du Boulay has been running at a local college for trainee primary school teachers. Primary school mathematics teaching is often a special problem partly because the teachers' own grip on the subject is none too firm. The aim of du Boulay's part of the Edinburgh project is to see whether LOGO learning has a function in helping primary teachers both to understand and to explain mathematics. That research is still at a preliminary stage.

But LOGO learning as a "catalyst" to communication is an important theme of the Edinburgh research. The particular term catalyst has been adopted by Sylvia Weir to describe the effect of LOGO learning on communications between an autistic child and the normal adults who work with him. Weir, working with Ricky Emmanuel, has found that allowing an autistic child to control the turtle from an array of buttons on a box stimulated him to communicate with them in a spontaneous way they had rarely seen in any other context.

She attributes this "humanising" effect to what she calls "shared relevance": the transparent connections between the pressing of the buttons and the movements of the turtle, and

between the turtle's actions and the child's own movements in space provide, in her view, a basis of shared relevance, or a common framework, within which the child and the adults can communicate. Building on a framework of shared knowledge is the basis for any intelligent and effective teaching, and educationists acknowledge that good teaching must depend on the teacher's being able to reach out to the child's position and lead him from there. LOGO learning may be a special help where the subnormality of the child makes the gap unusually large and the teacher's task unusually difficult.

Future of LOGO

But the commitment of the Edinburgh group is to find hard evidence for the possible benefits of computer-based teaching systems like LOGO. Most of the known methods of educational assessment will be used for that purpose, and Howe and O'Shea have added one new test specially designed to reflect some of the skills that LOGO is supposed to promote. O'Shea calls it the "articulation test", and it tests the ability of a child sitting on one side of an opaque screen to instruct his partner on the other side on how to construct a particular pattern from coloured blocks. That apparently simple problem has been known to reduce an untutored child to tears of inarticulate frustration, so the results should be interesting; but so far, they are only suggestive.

On what evidence there already is, what part are computer-based systems really likely to play in future education? Papert purports to believe that the future of education and computer-based systems are one and the same, and the next few decades will see the day when each child sits at home at his own computer terminal, without going to school at all. Expense he shrugs off on the grounds that electronics, unlike anything else, are getting exponentially cheaper (consider the cost of the pocket calculator five years ago and now). Nonetheless, equipment of any kind is expensive and computers and their peripherals will remain more expensive than books. And although the Edinburgh research team has been impressed by the way that Papert's educational insights have been borne out, from the practical point of view their attitude is both more conservative and more pragmatic than his.

First, they do not see in LOGO a means of disposing of the teacher: the idea is that such systems may make conventional teaching methods more effective, not that they should replace them. Second, experience is beginning to suggest that the most effective use of computer systems may turn out to be in the education of subnormal children or those with other special problems. If the Edinburgh group can be accused of having a vision, then it is of machines which foster human interactions, rather than making them obsolete. □

BRITAIN

Benn spells it out

Developments on the British energy front are fast and furious, with nuclear power capturing most attention. Chris Sherwell reports.

FAST moving sectors of the British economy are not in great abundance these days, but there is one that is beginning to behave in a manner representative of a seething hive of activity. Hardly surprising, for it is none other than the energy sector itself. And not a little of this is due to the increasingly voluble debate over nuclear power.

The arguments over whether Britain should have nuclear power already having been settled, the manner in which it is to be deployed and utilised is now at issue. That includes the question of timing, so there are tactical as well as strategic policy decisions to be taken. The latest involves the Steam Generating Heavy

Water Reactor (SGHWR), expenditure on which was deferred for a year in the latest round of government spending cuts.

The cut means a saving for 1977-78 of £40 million, and a reduction of £5 million on research for the project. That in turn has made the broadening debate on the future of the SGHWR either more pointless or more controversial, depending on your perspective. But it hasn't prevented a start to the hearings of the House of Commons Select Committee on Science and Technology on the subject. They began this week, Mr Anthony Wedgwood Benn, Secretary of State for Energy, answering questions.

Mr Benn was fresh from his discussions at the end of last week with Sir Arthur Hawkins, chairman of the Central Electricity Generating Board (CEGB), who supported the pressurised water reactor when a choice was being made in Britain in 1974 but has since, on Mr Benn's testimony, given every

assistance regarding the SGHWR. Sir Arthur was, however, expected to deploy cost estimates and electricity demand forecasts to advise against an immediate go-ahead, on the SGHWR or anything else. Mr Benn also had before him a report submitted within the past week from Sir John Hill, chairman of the UK Atomic Energy Authority (UKAEA), assessing the progress made so far on the SGHWR.

It was soon apparent that Mr Benn was concerned to make only four main points. One, as he pointed out at the beginning, the middle and the end of his evidence, the British Government has not yet reached a decision to cancel the SGHWR, nor even a preliminary view on the matter. Two, the decision through spending cuts to postpone for a year its commissioning, while possibly having consequences for the nuclear industry outside the SGHWR programme, was the product of deferment presenting itself as an option. This was in turn a result of "natural slippage" on the SGHWR's reference design and safety standards working in the same direction as the effect of

falling demand for power stations on energy forecasts.

Third, the initiative which resulted in the circumstances under which the SGHWR was now under scrutiny lay not with the government but with the UKAEA. Sir John Hill had approached Mr Benn and given his view on the SGHWR; Mr Benn had asked him to put this before the UKAEA board for its considered judgment, and that had been done.

It was this, in fact, which made the hearing the more revealing, for the report itself was actually supplied to the committee by Mr Benn before the hearings and was used as a basis for questioning Mr Benn. Inevitably, some of its details came tumbling out.

The UKAEA's view, it emerges, is that, because of lower electricity demand and public expenditure stringency, the SGHWR is less attractive than it was two years ago. The "consensus view" of the board is, with the exception of the historically enthusiastic South of Scotland Electric

city Board, that the SGHWR be replaced for the remaining thermal reactor programme by Pressurised Water Reactors (PWRs) or Advanced Gas Cooled Reactors (AGRs)—and that the AGR is the more promising, although operational experience is needed.

The UKAEA went further than this. Subject to a satisfactory outcome of the Nuclear Inspectorate's review of the PWR, there was a "strong argument" that the future needs of the nuclear industry could be met by the construction of PWRs under licence. This, Mr Benn freely acknowledged, would, if acted upon, constitute a reversal of the government's decision taken two years ago when his predecessor, Mr Varley, was at the Department of Energy. And, he said later, in the field of high technology one shouldn't "chop and change" once a decision was taken; he added reassuringly that he himself was "reluctant to be driven off".

The precise extent of his reluctance

was twice made quite plain when he made his fourth point. This outlined his response thus far to the UKAEA, and it amounts to a warning. If the SGHWR programme is dropped, says Mr Benn, it could be a setback to the country's whole reactor programme. A decision to go for the PWR would mean re-commencing the whole effort to produce a reference design to meet UK conditions, and thus more delays, and even then it would not certainly provide a solution. It would also set aside the past two years' work, and previous work carried out at Winfrith in Dorset.

Moreover, this would amount to a statement from the UKAEA that it had lost confidence in its own system. That would in turn rebound and damage the credibility not just of the UKAEA, but of the systems it sponsors, including the fast breeder, and of the whole nuclear idea. The nuclear issue, in short, might be thrown right back into the public arena, "where it may belong". □

FRANCE

Staying nuclear

France's nuclear programme has come in for some domestic criticism recently, but looks like pressing ahead. A Correspondent reports

It is August, France is on holiday, and that has brought a degree of quiescence to town and country. There is even time for some sort of break from politics, or at least from certain political issues. But it remains uncertain whether, come September, one issue in particular will have been allowed completely to die away. The issue is energy—specifically nuclear energy. Specifically the fast breeder reactor. For, despite all the impressions conveyed abroad, the priority France gives to her nuclear programme in her overall energy strategy does not go totally unchallenged. And the priority given within that to the breeder reactor has not escaped attention.

The whole issue finally blew up early last month, at a place called Creys-Malville, which is near Lyons and on the Rhône. Under construction there is perhaps the most advanced reactor of its type in the world. Superphénix, it is called, and it is expected to be on stream in the early 1980s—all 1,200 MW of it. Like Britain, France already has a prototype breeder reactor, a 250 MW plant known as Phénix situated on the Rhône at Marcoule. But while Britain agonises about whether

to go ahead with a demonstration commercial-scale breeder, France is on her way—or looked to be until last month, when there was a new development.

It was Europe's anti-nuclear lobby which stepped in. Superphénix, already the object of trade union protests, became the butt of criticism from ecologists and environmentalists as well. They came in hundreds and camped outside the site where workmen toiled. The jamboree lasted several days. The way it was brought to an end, however, aroused even more indignation than perhaps the construction itself: riot police moved in with tear gas and truncheons to evict the demonstrators, and some of the protesters were hurt.

The criticism in France, as in other industrialised countries contemplating the use of the fast breeder reactor, is emotive inasmuch as it rides on the back of fear, particularly about the implications of the large-scale production of plutonium. It is not less rational, or less important, for that. But its form is characteristic. So, too, is the response from Electricité de France (EDF) and other interests within the French nuclear industry, and from the government too. Just look at France's energy position, they say.

Imports account for something like 75% of the country's energy requirements, a dependence which France must seek to reduce, if only for strategic reasons. What is more, the argument runs, unlike Britain, the country has no

oil, and its coal reserves don't compare with those of Britain or Germany. That means nuclear power is likely to make economic sense as well. And the alternative threat of an energy shortage embodies implications as alarming as nuclear power for people's lives.

Of course France, like other industrialised countries, has been forced to revise its energy demand forecasts in light of general economic developments. Energy use last year, for example, dropped by over 5%, more than the fall in national product, and there is increasing emphasis on energy conservation. A fair amount of encouragement is also given to research and development on new sources of energy.

But no one seriously doubts that France needs nuclear power now and will need it even more in the future. Even a special commission examining energy policy in France, formed partly in response to concern expressed last year by 400 academics at the increasing use of nuclear power, has not recommended any slowdown. Following a review of the nuclear programme earlier this year plans for power capacity over the next few years includes construction of 6,000 MW of nuclear power this year and next, and 5,000 MW in 1978. The change this represents on the original programme is marginal, and, despite the protests, the talk is still of 40,000–45,000 MW of nuclear power 1985. By that time most of the country's electricity will be nuclear generated and a substantial proportion of its total energy will be coming from nuclear plants.

All of this, moreover, will be taking place within the context of a thoroughly reorganised nuclear industry. Plans for conventional plant will continue to focus on the pressurised water reactors. Looking ahead, France expects that the new generation of fast breeders will be needed in the 1980s. That is why Superphénix received the go-ahead earlier this year. And the fact that France has secured the cooperation on the project of both Italy and Germany while retaining a 51% interest for EDF is of perhaps greater

consequence to many of those awaiting a British decision on the fast breeder than the debate on its implications which is now growing in all the industrial countries.

● The final contract for the controversial Franco-South African deal involving the sale by a French consortium of two nuclear power plants to the South African Electricity Supply Commission (Escom) were not signed by the deadline of July 31 last week following the development of a complication, apparently over safeguards

attaching to the deal. A South African delegation left Paris after 24 hours of talks without having signed anything, and although French spokesmen sounded confident this raised doubts about whether the deal would go through. It now appears that an agreement concerning safeguards was later initialled in Paris and will be submitted to the International Atomic Energy Agency and Euratom. It is thought that once this is accepted, it will pave the way for the signing of the main contracts.

WEST GERMANY

Exporting for survival

West Germany's nuclear industry is not becoming any less involved in the politically sensitive area of exports. A Correspondent reports.

FURTHER developments in the field of nuclear exports have come with news of additional moves from West Germany exploiting this increasingly lucrative market. Reports quoting West German Economics Ministry officials and spokesmen for the country's leading power station constructor, Kraftwerk Union, indicate that West Germany has been clearing the way with Nigeria for negotiations to begin on the first sale of a 500-600 MW German nuclear plant to a black African country.

The news coincides with the visit to West Germany of the Nigerian Prime Minister, General Yardua. It also comes just a week after Brazil had signed delivery contracts with Kraftwerk Union for the construction of two nuclear reactors as part of

last year's government-to-government agreement, and less than a month after the signing in Tehran of the contract between Kraftwerk Union and the Atomic Energy Authority of Iran for the turnkey supply of two 1,200 MW pressurised water reactors.

With the signing of the latter contract, West Germany has become the first western enterprise actually to conclude a nuclear contract with Iran. The reactors, which are to be built near the city of Bushehr on the shore of the Persian Gulf, were begun this time last year after a letter of intent was issued to Kraftwerk Union in November 1974. Of the 3,800 employees presently on site, about 600 are German expatriates.

Kraftwerk Union describes the plants it exports as the most reliable reactor systems, and generally cites the Biblis plant in Germany in support. In fact, as Rheinisch-Westfälische Elektrizitätswerk (RWE) confirmed last week, that station has now revealed technical problems in the course of a planned shutdown, and the length of the closure

is now likely to be doubled to four months.

What this means in terms of costs for Kraftwerk Union is uncertain, but it does not expect to operate at break-even point until the 1980s anyway. The star of Germany's nuclear industry, the company is crucially dependent on exports to supplement the home market and so render possible the economies of scale necessary to compete favourably with the USA. But some overseas contracts were lost in the first half of 1975. These included the plan for a nuclear power station at Kaliningrad on the Soviet-Polish border, which fell through because of East German objections concerning the supply of electricity, and deals with South Africa, Sweden and Switzerland.

The company also appears to be finding that permits are now taking much longer to obtain, and greater safety measures, among other things, are extending the construction time for the average plant. Germany has its own protectors of the environment too, keen to prevent (or at least delay) construction of plants beyond the ten or so already in operation. □

SWEDEN

Suffering without support

Wendy Barnaby reports from Stockholm on the state of basic technical research in Sweden

To live in Sweden is to be impressed by the sophistication of the country's technology: a level of accomplishment which thrives on extensive research and development. There is, however, one part of this body of excellence which is ailing. Basic technical research is, in fact, close to death.

Nobody seems to know exactly what basic technical research is, but everyone agrees that it is conducted for the extension of knowledge, without any specific application in view—although

it may of course be used if the necessary techniques are developed. It is also differentiated from pure fundamental research, which has no possible or likely practical application and which is supported in Sweden by the Natural Science Research Council. Opinion is unanimous, too, on the extent to which basic technical research is presently being supported. In the words of a spokesman at the Academy of Engineering Sciences, it is being given "no money at all".

This is hardly an exaggeration. Its needs used to be met by the State Technical Research Council. This body was disbanded in 1968 and replaced by the Swedish Board for Technical Development (STU) whose emphasis has

been on supporting applied research, specifically of the sort that will further innovation and technical expertise in Swedish industry. Including governmental and industrial outlays, Sweden spends about Sk4,000 million (about \$900 million) annually on research and development. For the 1975-76 financial year, STU's budget amounted to about \$56 million, of which—according to a spokesman there—about \$10 million was devoted to basic technical research.

Or, rather, to some types of that research. For there has been a re-orientation of grant-giving to favour certain areas of "big" science—space and elementary particle research, for example. This has resulted in fewer numbers of bigger grants being given in these areas, rather than a multitude of smaller grants covering a broader

range. Dr Gösta Lagermalm, STU's senior policy adviser, agrees that because of this change of emphasis, the complaints of physicists, chemists and biologists trying to pursue "small" basic technical research are justified.

Part of the work of a governmental commission set up in 1972 to survey Swedish research councils and make suggestions for changes in their organisation was to consider this particular problem. During the commission's investigations, STU was asked to indicate those projects supported by its 1971-72 budget which had had their support reduced or cut off entirely in succeeding budgets because they were too much oriented towards basic technical research. STU replied that in 1971-72, it had given about \$1.2 million to 49 such projects. This fell in 1972-73 to just over \$650,000 for 31 projects, only four of which would be kept on for 1973-74—all for the last time.

But approaching the problem through statistics does not really give the feel of it. Neither can it be gauged by comparing the number of applications for basic technical research grants received with those approved. As one professor at the Institute of Technology explained, colleagues of his who have been refused grants no longer apply for them, even though they still want to pursue their work and believe that it is more important for the country's technical development than the short term projects they are forced to switch to. Another said that some colleagues he knew had tried to get funds by dressing their basic technical research applications to make them look like projects which would yield practicable results quickly.

Various propositions have been put forward to find a new home for basic technical research. Some have suggested that the Natural Science Research Council should take on the job, others that STU should be given more funds in order to bring the research under its umbrella. Another suggestion was that this sort of research should have its own dispensing body. The governmental commission recommended last year that STU assume full responsibility for "support for technical research of a basic nature". This would mean setting up another grant-giving body within the framework of STU, a task which seems hardly likely to be undertaken in view of the fact that yet another commission is presently studying the aims and structure of STU itself, and is not due to report for another 18 months or so.

Thus, while the planners plan, part of the activity they are meant to be stimulating is simply being strangled, with the limiting of scientific enquiry and the lowering of morale of those academics concerned as the result. □

CANADA

Council rift over report

The Science Council of Canada received a little more public attention over a recent incident than it wanted. David Spurgeon reports from Ottawa

THE well-known French-Canadian science broadcaster Fernand Seguin resigned from the Science Council recently when it would not publish his dissenting views on a council report on population, technology and resources. The report calls for a sharp reduction in immigration to slow Canada's population growth and maintain its high standard of living. It also proposes that Canada should use high technology to increase its food production and exports of food and raw materials.

Mr Seguin contended that the large increases in food exports proposed would help create a small club of privileged countries and would support the US strategy which seeks to use food as a political weapon in a way that the OPEC countries have used their oil. He maintained that the high technology called for in the report would require massive participation by multinational companies, a large-scale influx of foreign capital, and construction of more than 100 nuclear power plants in Canada. The upshot would be that Canada would align itself with US "food imperialism", and "hunger-stricken countries would have to come and eat from our hand".

Another council member, Dr Ursula Franklin, professor of metallurgy and materials science at the University of Toronto, also disagreed with the council report. She re-assessed her position on the council as a result of the refusal to issue a minority report, but later decided not to resign, saying she would continue her participation in the council's project on the Conserver Society, of which she is head. (The Conserver Society is one of the three themes around which the council has recently structured its programme. The other two themes are technological independence and industrial strategy, and the health of the Canadian scientific community.)

In a newspaper interview ahead of the council study's publication, the study's chairman (and a former council chairman), Dr Omond Solandt, proposed cutting immigration to Canada from last year's 143,000 to an annual net figure of 50,000 to produce a population of 28 to 30 million by the end of the century (present population is about 22 million). He argued that Canada can only export food if it does not import people. Furthermore, he

said that Canada could feed four or five times as many people by exporting grain as it could if they lived in this country, since people in developing nations consume only about 400 pounds of grain a year compared to 2,000 pounds consumed by North Americans.

Energy would also be a problem, said Dr Solandt, because Canadians consume more energy than do people in developing nations. "If we brought in another 20 million, it would completely disrupt the economy," he said. "We probably wouldn't be able to feed the people brought in, and we certainly wouldn't be able to keep them warm." Dr Franklin has contended that it is not possible for a country with Canada's small population to increase food and raw material production greatly without high technology, and says this will mean that Canada will increasingly have to sell out to foreign capital. It would also require highly centralised planning.

In Dr Franklin's mind, the problem with the Science Council report is not so much with its population targets as with these problems of technology. Immigration levels can be adjusted up and down, but high technology is irreversible, she maintains. It also involves environmental and social costs, and she says council members have demonstrated an unwillingness to come to terms with the inevitable social problems.

The council chairman, Josef Kates, said the minority report was refused because it had nothing constructive to offer. French-Canadian reporters raised the issue at a press conference held to release the council's annual report only a day or two after the controversy surfaced and vice-chairman Claude Fortier, director of the department of physiology at Laval University, acknowledged that the council has published minority reports in the past.

The council accepts the right of members to differ with a majority report, but the council reserves the right to decide the mode of transmission of the minority report, he said. Sometimes this is simply a note, sometimes it is done in the letter of transmittal of the report to the Minister of State for Science and Technology (the method to be followed in this case). Dr Fortier said no report of council had had more discussion than this one (a remark that echoed Dr Solandt's comment that the study was the most difficult ever undertaken by the council). Both Dr Fortier and executive director J. J. Shepherd said they regretted Mr Seguin's departure. □

IN BRIEF

Sea Law, round four

The Law of the Sea Conference convened in New York for another seven-week session this week surrounded by a major new uncertainty, the Presidential campaign in the United States, but with some other problems effectively sidestepped by the sprinkling (not to say, flood) of unilateral declarations of 200-mile fishing limits made in the past few months. The EEC countries have now declared their joint intent to introduce a community fishing limit, even if the conference does not come up with a proper agreement. It is, however, the wider question of how to deal with mineral resources on other parts of the sea bed that is likely to exercise the New York session most. How are these resources to be exploited "for the benefit of mankind as a whole" (to coin a conference phrase)?

And would the proposed International Seabed Authority actually wrap up every manganese nodule so inextricably in red tape as to make a good principle impossible to work out in practice? Any real decisions are likely to have to await a fifth round of conference—and the result of the US election in November.

What's happening on Mars?

Two of the three experiments carried on the Viking lander and designed to detect reactions in the soil characteristic of living organisms have so far yielded data. One, measuring the conversion of radioactively-labelled nutrients to labelled carbon dioxide, showed an initial rapid burst of activity which has since died down to almost nothing. In another, the soil released large amounts

of oxygen when initially dampened with water, even before the experiment proper (of adding a concentrated 'chicken soup' of nutrients) had begun, but nothing has happened since.

The best guess at the moment is that the Martian soil contains large amounts of bound oxygen, a not improbable speculation as the soil is rich in iron minerals which in some circumstances can readily bind oxygen under the influence of ultraviolet light. This would accord with earlier speculation that oxygen and hydrogen originating from water might be trapped in the iron minerals on Mars. So far it looks as though Mars may be the Mecca of the soil chemist (since no known soil on Earth has ever produced similar reactions, although the search is now on amongst desert soils) but that the question of life there remains as much of an enigma as ever.

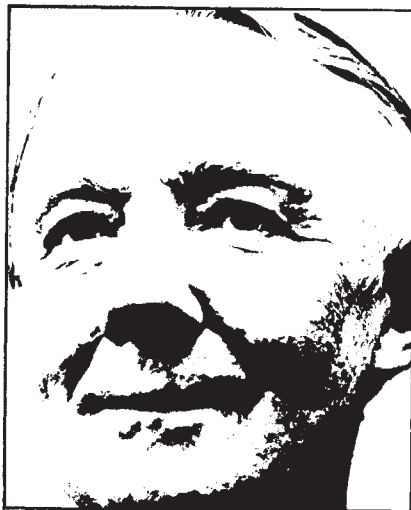
PLANNING must be based on the best possible forecasts of future developments. Unfortunately the best is generally not very good. Dr Walter Marshall told a conference on Renewable Sources of Energy organised recently by the Royal Society of Arts that the only certain thing about forecasts of energy use was that they would always prove to be wrong. Thus the Central Electricity Generating Board's projections made in the early 1960s overestimated the peak load for current used in 1975 by some 40%, which makes one suspicious of their figure foretelling a doubling or even trebling of the use of electricity in the period up to the year 2,000.

Population projections are even more difficult. Thus one report published shortly before the 1939-45 war, when England and Wales had a population of 41 million, suggested that this might fall to 31 million by 1975, and that the chances of a population increase were remote. In fact numbers increased by 8 million. The most recent figures issued by the Office of Population Censuses and Surveys suggest that the present trend to ZPG (zero population growth) will suffer a modest reverse after 1981, and that numbers in England and Wales will grow by about 1½ million by 1991. It will be interesting for those who survive to see what really happens.

Forecasting traffic densities, on which future road programmes are based, is an equally chancy business. Until recently the Department of the Environment decreed that their figures produced by the Road Research Laboratory must not be questioned.

This arrogant attitude has at length been somewhat relaxed, but an enormous increase in the number of private cars and of their use is still

Forecasting error



KENNETH MELLANBY

postulated. Yet there is little reason to believe that traffic projections are any more accurate than those for power or population.

Unfortunately many people seem to think that forecasting is becoming increasingly scientific, and that the use of computers is making the results more reliable. It is true that a great deal of mathematical skill has gone into the study of population trends, energy use and traffic census, and that the calculations arising therefrom have sometimes been speeded up by suitable computer programs, but unfortunately there has been no

improvement in obtaining the data used. The whole process has too often been one of "garbage in, garbage out". There are so many unknown and uncontrolled variables that it might be better to rely on crude sums done on the back of envelopes, than to use elegant calculations and expensive machinery which give a spurious illusion of scientific accuracy.

Some errors cannot be eliminated. We could foretell the birthrate accurately for about eight months from any date, and, provided there is no war or major epidemic, estimates of death rates and total population are foreseeable for a similar period. Forecasts for longer periods are simply guesses, affected by social factors which, so far, have defied accurate quantification. Only some form of rigid totalitarianism could improve the statistics.

Unfortunately some projections have a built-in source of error. Our government is committed to the idea of economic growth. Scientists in public employment appear to be expected to assume that this growth will take place, and that there will be increased energy consumption, more consumer goods, more travel and a growing "environmental impact". It is assumed that government policy will stimulate this growth in the economy. Some believe this to be likely, others describe schemes intended to produce growth as "pouring out public funds like water, to create lakes in which lame ducks will sink". It may be impossible to keep politics out of forecasting, but political factors should be identified and not concealed.

correspondence

Lithium supplies

SIR,—We fully support the contention of Walton and Spooner (June 17, pages 533–5) that there is a pressing need to identify further resources of lithium, but would take issue with them on a few points.

In Table 2 some 50% of their total resources derives from North American brine deposits. However, J. D. Vine (*Journ. Research U.S. Geol. Surv.*, 3, 4, 479–85; 1975) recently emphasised that the amount of *recoverable* lithium at Silver Peak is, in fact, lower than the published reserves by a factor of 10. Should a similar factor apply to other brine deposits the shortfall between Western world reserves and projected consumption becomes critically magnified.

Our present, limited, understanding of the geochemistry of lithium suggests that more small brine and pegmatite sources may be discovered in the developed countries. Even so, the best prospects for large reserves lie in Africa and South America, sources which for various reasons may not automatically be available to the technologically advanced Western nations. We may be obliged to rely, therefore, on known smaller domestic deposits and on exploration and development of new types of lithium deposit.

Walton and Spooner state that the European countries “possess no presently identified significant lithium resources”: though true of reserves it is not so of resources. Small lithium pegmatites and lithia brine flows are known in several European countries, including Great Britain, but none is exploited at present. Given suitable financial inducement to development, they could make a valuable though comparatively small contribution to our requirements.

Of more significance are the lithium-rich portions of the Variscan granites, in which lithium occurs largely in “lithionite” mica. In Cornwall, for example, parts of just such a major lithium resource have been affected by kaolinisation and some of the lithionite could be extracted during the working of china clay. As yet the distribution of lithionite-granite in the Cornubian batholith is imperfectly known and we have only sparse information on the variations in lithium content and none on isotopic composition. Nevertheless, exploitation of zinnwaldite mica from granites of the Czechoslovakian Erzgebirge, albeit under a different economic philosophy, indicates that this type of deposit may have an important role to play in the supply of future lithium requirements and argues for a direct programme of geological and chemical engineering research into the lithium potentialities in the Western European Variscides.

Yours faithfully,
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Bran and sawdust

SIR,—It was suggested by Dr Jukes (May 13, page 92) that bran and sawdust constitute a safe diet. This is not so. There is evidence (Mackarness, *Not All in the Mind* (Pan; 1976)) that, on an evolutionary time scale, cereal is a recent addition to our diet, one to which we have not yet become adapted, and a Stone Age pre-cereal diet was recommended.

As for sawdust, it is highly likely that timber is carcinogenic: wood workers have a high incidence of nasal adenocarcinoma (Acheson *et al.*, *Brit. J. Industr. Med.*, 29, 21; 1972), while mice bedded on wood shavings show an increase in so-called “spontaneous” tumors (Sabine *et al.*, *J. Natl Cancer Inst.*, 50, 1237; 1973).

It is high time to accept the fact that nothing whatever is completely safe.

Yours faithfully,
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Canned crab

SIR,—In 1959 Ecuador declared the unoccupied lands of the Galapagos Archipelago a national park; by 1968 a department of national parks and wildlife was created to administer the park. The national park service started collecting an entrance fee in 1972 that, in part, is used to finance conservation programs in the islands. Although conservation efforts have grown, tourism has grown more spectacularly. For instance, since 1969 the number of tourist boats have more than doubled.

There is currently an effort to extend

the national park to protect the marine life near the Galapagos shores. It will be difficult to regulate the fishing but this may become necessary. Packing boats started to visit the islands regularly in 1972, allowing fishermen to go farther and stay out longer. Many of the endemic species such as the Galapagos penguin are dependent on small fish which could become commercially valuable. The Galapagos penguin is subject to total nesting failures suggesting that competing pressure from humans could, over a period of time, reduce their population.

Dramatic decreases in species number or composition will probably be detected but subtle changes could go unremarked. In 1972 when no more than 80 tourists were visiting Pta. Espinosa, Fernandina weekly, a hermit crab with a discarded film can marched through my camp in July and August. Our camp site was a mile from the tourist landing and we had no similar film canisters. The hermit crab's aluminium home should have heated up rapidly in the hot equatorial sun, which may be why the crab stayed in the shade of the mangroves. Pesticides have been found in almost every remote corner of the world; the film canister may be next.

Yours faithfully,
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news and views

Anti-idiotypic antibodies: a clinical future?

from L. Brent and P. J. Kilshaw

ONE of the most recent concepts emerging from the witches brew of present-day immunology is the exciting possibility that antibodies directed against the receptor sites of other antibodies may have a role in regulating the immune response.

It is more than a decade ago that Oudin and Michel (*C.r. hebd. Acad. Sci. Paris*, **257**, 805; 1963) observed that antibody receptor sites (which bind antigens in a highly specific manner) themselves bear antigenic determinants and that these 'idiotypic' determinants (or idiotypes) can evoke antibody production in animals belonging to the same species. Idiotypes owe their existence to the unique configuration of the variable region of specific antibody molecules, and they should not be confused with 'allotypes'—again first described by Oudin—which are antigenic specificities present in the immunoglobulins and other proteins of different groups of individuals of the same species and determined by different alleles of the same gene. The vast repertoire of antibody receptor sites within a single individual is associated with a great heterogeneity of idiotypic determinants, and so the production of antibodies with combining sites for the stimulating antigen is matched by the appearance of a limited range of idiotypes. The observation that antibodies can combine with the receptor sites of other antibodies led Jerne (*Ann. Immun. Inst. Pasteur*, **125C**, 373; 1974) to suggest that the production of anti-idiotypic antibodies might be a normal event that modulates the degree and duration of the response. This could theoretically be the case provided that lymphocytes carry receptor sites on their surface identical to those on immunoglobulin molecules: by combining with these receptors, anti-idiotypic antibodies could prevent contact between lymphocytes and their specific antigens or even cause cell death, thus limiting the immune response. Such a mechanism would presumably depend on the replication of specific idiotypes up to or beyond the threshold at which antibody production against them ensues;

that is, by an increase in the antibody titre in the case of B cell responses, and for T cell responses, an increase in the number of cells with specific receptors. At present there is little direct experimental evidence to support this fascinating theory, but over the past few years there have been a number of successful attempts to suppress both humoral and cellular responses by passively administering anti-idiotypic antibodies or by actively stimulating their production.

The urgent need for a specific form of immunosuppression in clinical transplantation has provided an additional incentive for the study of anti-idiotypic antibodies, using T cell receptors or alloantibodies directed against specific transplantation antigens. Ramseier and Lindenmann (*Path. Microbiol.*, **34**, 379; 1969) were the first in the field: they reasoned that if an F_1 hybrid mouse resulting from the mating of animals of two inbred strains (say A and B) is injected with lymphocytes from one of the parental strains (say A), the only antigenic determinants present on the injected cells recognised as foreign by the F_1 recipient would be receptors for antigens of strain B. (A mouse which possesses both A and B antigens on its cells will not have receptors for either). In these circumstances the production of anti-(anti-B) antibodies could be expected. Using a rather indirect and cumbersome test, the PAR (Product of Antigenic Recognition) assay, Ramseier and Lindenmann (see *Transplant. Rev.*, **10**, 57; 1972) showed that sera taken from animals sensitised in this manner were capable of suppressing the *in vitro* reactivity of strain A lymphocytes against alloantigens of the B strain. The immunosuppressive moiety responsible for this effect was not, however, fully characterised.

More recently, Binz, Wigzell and their colleagues (see Binz, Kimura and Wigzell, *Scand. J. Immun.*, **4**, 413; 1975) have adopted a similar approach using the inbred rat strains Lewis and DA. They found that anti-receptor site antibodies could be prepared optimally by injecting purified T (thymus-dependent) lymphocytes from one of

the parental strains (Lewis say) into F_1 hybrid recipients. In this case then, sera taken from the hybrids would specifically abolish the response of Lewis lymphocytes against DA antigens, and this has been shown with the aid of graft-versus-host and mixed lymphocyte culture reactions. These assays test the ability of lymphocytes to recognise and respond to specific alloantigens. Furthermore the F_1 hybrid antisera formed a precipitate with alloantibodies of the appropriate specificity and they agglutinated alloantibody-coated red cells.

Anti-idiotypic antibodies might be expected to provide a useful tool for characterising the elusive T cell receptors for antigens of various kinds. By absorbing putative anti-receptor site antibodies with purified T cells or with alloantibodies, Binz and Wigzell (*J. exp. Med.*, **142**, 197; 1975) were able to show that the idiotypes on the T cell receptors were closely similar to, or identical with, those present within the combining sites of immunoglobulin molecules. Further, they succeeded in identifying receptors from both T and B cells in the body fluids of normal Lewis rats.

In a paper published in *Nature* recently Binz and Wigzell (*Nature*, **262**, 294; 1976) describe the effects of an anti-idiotypic response on the survival of allogeneic rat skin grafts. To evoke the anti-idiotypic response they sensitised Lewis rats with Lewis receptors specific for antigens of the DA strain. The receptors were isolated from the serum of normal Lewis rats with the aid of an immunosorbent consisting of antibodies specific for Lewis anti-DA idiotypes coupled to sepharose particles, dissociated from the immunosorbent, treated with glutaraldehyde (this causes cross-linking and increases immunogenicity) and injected in Freund's adjuvant into four Lewis rats. This procedure was repeated three weeks later. DA skin grafts transplanted to these animals 19 days later had a mean survival time of 30 days, one of them surviving for 38 days, compared with 10 days in the untreated controls. (This observation was

confirmed in a larger group of recipients, in which the mean survival time proved to be somewhat less, 24 days.) The partial unresponsiveness was specific in that grafts from a third strain (BN) transplanted at the same time were rejected with a mean survival time of 12 days. The lymphocytes of the four immunised Lewis rats were tested for MLC reactivity before skin transplantation and found to be non-reactive to DA but fully responsive to BN antigens. Some time after they had rejected their skin grafts the sera from these rats were subjected to rigorous tests which established without doubt that anti-idiotypic antibodies were still present in significant amounts—a finding that received support from the observation that these animals suffered from an impairment of alloantibody production when they were challenged with (DA×Lewis) F_1 hybrid lymphocytes.

Binz and Wigzell propose that anti-idiotypic antibodies caused the physical elimination of cell clones which were specifically reactive to DA antigens. Whilst the results are impressive and the

authors' interpretation entirely plausible a note of caution must nevertheless be sounded, especially in relation to the clinical application suggested by Binz and Wigzell. First, the complexity of the manipulations involved is considerable. Second, investigations involving the immunoregulatory powers of anti-idiotypic antibodies in transplantation have been largely dominated by the Lewis and DA rat strains, and it is now vital to show that the phenomenon extends to other strains and to other species. Third, the prolongation of graft survival achieved in these experiments, though considerable, is strictly limited: in view of the apparent presence of anti-idiotypic antibodies long after the grafts had been rejected one may well wonder why survival was not more prolonged or even permanent. It would seem that anti-idiotypic antibodies alone are not sufficient to guarantee complete unresponsiveness. Finally, the active production of these antibodies depends on pretreatment of graft recipients; even if it were possible to apply the rat methodology to human graft recipients (and there must be

considerable doubt about that) pretreatment would not be practicable in the transplantation of cadaveric grafts, that is, in a situation in which the intercession of a specific form of immunosuppression is most needed. Perhaps the most likely application to man will be in the control of autoimmune diseases.

Despite these reservations the data of Binz and Wigzell are clearly of the greatest importance, if only because of their impact on theories of antibody formation and control. Anti-idiotypic antibodies with specific immunoregulatory properties have been described in other systems, and these have so far always involved antigens that evoke antibodies of very narrow clonality (that is with a narrow range of idiotype determinants), such as phosphoryl choline (Cosenza, *Eur. J. Immun.*, **6**, 114; 1976) and group A streptococcal carbohydrate (Eichmann, *Eur. J. Immun.*, **5**, 511; 1975). Time alone will tell whether this special feature is a prerequisite for the production and immunoregulatory properties of anti-idiotypic antibodies. □

Associated production of charm

from W. T. Toner

A NEW heavy particle with two properties characteristic of the charm scheme (see *News and Views*, **259**, 622; 1976) has been discovered by the Stanford-Berkeley collaboration working on the SPEAR storage ring at Stanford in California (Goldhaber *et al.*, *Phys. Rev. Lett.*, August 2, 1976). It was observed in decay modes containing K mesons and seems always to be produced in association with one or other of two heavier particles whose presence is inferred from indirect evidence.

When the same group discovered the ψ family and showed that the unexpectedly large rate for the annihilation of high energy electrons and positrons began suddenly at the point where the ψ series changed from having uniquely narrow widths to a more normal shape, most particle physicists confidently assumed that the excess was due to the copious production of charmed particles into which the heavier ψ 's could decay rapidly: it should be as easy to create two oppositely charmed particles out of the annihilation energy as two charged particles. There should be no difficulty in finding them since theorists predicted frequent decays involving K mesons.

Months later when the only reports from Stanford were of fruitless searches, people began to find excuses.

Easily identified decay modes containing K mesons and no neutral particles might be rarer than one had thought. It now seems that everyone had underestimated the amount of careful work needed to find even the most obvious decays. In fact, with hindsight, one can find some evidence for the new particle in the previous negative report (BoyarSKI *et al.*, *Phys. Rev. Lett.*, **35**, 196; 1975).

In the experiment every electron-positron annihilation event which results in two or more charged particles being detected is recorded. After cases with electrons or mu-mesons are eliminated, each event is analysed to test the hypothesis that a particle was produced which decayed into a π and a K meson. Charmed lives, though unusually long, should still be much too short for the charmed particles themselves to leave tracks; only π and K mesons or protons (anti-protons if negatively charged) can be recorded.

From a rough determination of the velocity of each particle and a precise measure of its momentum, the analysis programme first computes the relative probability that the particle was a π meson, a K meson or a proton so that a precise mass can be assigned. When the decision is ambiguous between K and π , both possibilities are included

and the event is analysed as if it were two (or more) different events each with an appropriate fractional statistical weight. The energy of each particle can now be calculated accurately from Einstein's relation between mass (m) energy (E), momentum (p) and the velocity of light (c): $E^2 - p^2c^2 = m^2c^4$.

If an event contains a π and a K meson of opposite charge their energies and momenta are added and the "effective mass" of the pair is calculated. The event is next treated as if no other measurements were available (very few events are complete) and the energy and momentum of the "missing" system containing all other particles produced is found by subtraction from the values given by the storage ring parameters for the annihilation as a whole. The mass of the system recoiling against the pair, known loosely as the "missing mass", is now calculated from the mass-energy relation. The analysis is repeated for any other possible ($\pi^\pm K^\mp$) pairing.

"Effective" and "missing" mass histograms are built up with results from many thousands of individual annihilations. In most cases there is no relationship between the paired particles. Such random pairings give the histograms a smooth shape on which are superimposed peaks at distinct

masses corresponding to correct pairings of the decay products of real particles.

The SPEAR effective mass histogram shows an unmistakable peak at a mass of $1.86 \text{ GeV}/c^2$. If the missing mass histogram is restricted to events with a π -K effective mass of this value, a strong signal is seen which more recent data show to be two peaks with "missing masses" of 2.02 and $2.15 \text{ GeV}/c^2$. No decay modes have as yet been reported for these heavy recoils. The widths of the peaks are unusually small for particles with such large masses but the experimental resolution is not fine enough to class them apart on the grounds of width alone. The data strongly suggest but do not yet prove that all non-random π -K pairs with a mass of $1.86 \text{ GeV}/c^2$ are produced in association with recoils in the high mass peaks. Similar features are found in the analysis of $(K^\pm \pi^\pm \pi^\mp \pi^\mp)$ systems.

It is very hard indeed to explain these observations in terms of "just" three more ordinary particles. Besides, were they so they should have been seen in many experiments at proton accelerators. The associated production of charmed particles is the only natural explanation.

Progress in the spectroscopy of charmed particles should be rapid now that three masses and two decay modes have been identified. They can be used as tracers to help identify the cascade decays of heavier charmed particles which should contain them. If these particles are the same as those seen decaying with the emission of positrons in the neutrino experiments (*News and Views, loc. cit.*), events not containing positrons should have $(K\pi)$ pairs and $(K\pi\pi\pi)$ systems at this mass in relative amounts predictable from the SPEAR data. And so on.

The large and extraordinarily productive group working at SPEAR will continue their experiments. If they go on at the present rate the new particles will soon outnumber the experimenters. Further results from the SPEAR group were presented at the recent Tbilisi conference a report of which will be appearing in a forthcoming issue.

Quasi-superheavy nuclei

from P. E. Hodgson

THE recent discovery of superheavy nuclei in monazite, a mineral from Madagascar (*News and Views*, **261**, 627; 1976; *Phys. Rev. Lett.*, **37**, 11; 1976) will stimulate further interest in the transient quasi-superheavy nuclei

that are formed briefly during collisions of heavy ions.

In such collisions, the heavy ions remain near each other for a short time that depends on their relative velocity. Two lead nuclei, for example, one stationary and the other moving with 10 MeV per nucleon remain within two nuclear diameters of each other for about $3 \times 10^{-22} \text{ s}$. Since this separation is far smaller than the radius of the innermost electron orbit there is no difference, from the point of view of the electrons, between two colliding lead nuclei and the single nucleus that would be formed by their fusion.

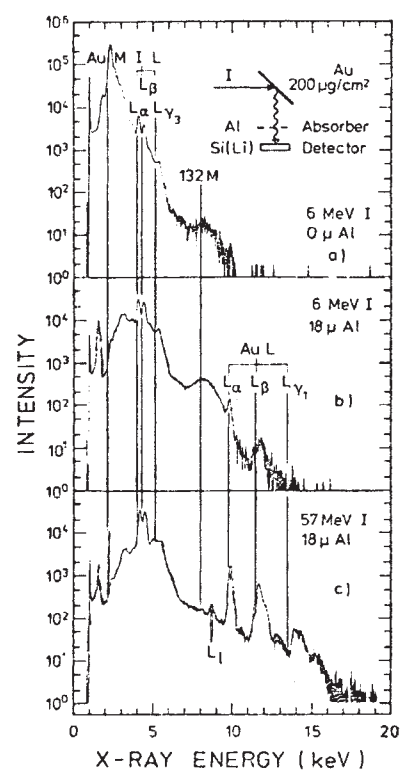
The electrons in the colliding ions are in the orbits appropriate to lead, and when the two nuclei collide the two electron clouds are in violent interaction. If now the nuclear interaction time is long enough these electron clouds can rearrange themselves in the configuration corresponding to the electron orbits of the combined super-heavy nucleus. This system of two colliding nuclei and the electron cloud of the combined nucleus is called a quasi-superheavy nucleus.

The likelihood of formation of such nuclei depends on a delicate balance of interaction and rearrangement times, so that they can only exist, if at all, for a limited range of relative velocity of the colliding ions. If this velocity is too low, the nuclei will not be able sufficiently to overcome their mutual electrostatic repulsion to appear to their electrons as a single entity. If on the other hand the velocity is too high, they will not remain in close contact long enough for the electrons to rearrange themselves into the electronic configuration of the combined system.

Calculations have been made of these limiting velocities, and it is found that the ratio of the upper to the lower velocity is about 0.13, independent of the atomic number or the electron shell. Thus for all electron shells there is a range of velocities for which the system can be considered a quasi-atom. The optimum distance of closest approach which corresponds to the greatest lifetime of the quasi-atom gives $T_{\text{max}} = (n^3/Z^2) \times 10^{-18} \text{ seconds}$ where n is the principal quantum number of the electron orbit and Z the nuclear charge.

One way of showing that a quasi-atom has been formed is to detect one or more of the characteristic X rays due to inner shell transitions and to verify that they have the required frequencies. This is the classical method due to Moseley for measuring the nuclear charge. For such transitions to take place it is first necessary to form a vacancy by removing an electron from an inner shell.

This can be done by forming a vacancy in the projectile and then



X-ray spectra from a gold target bombarded with iodine. The broad peak around 8 keV is attributed to X rays from the combined system with $Z=132$.

transferring it to the combined system. Such vacancies can be produced in heavy ion collisions mainly by electron promotion, in which some of the energy of the interaction is changed to excitation energy. The vacancy then has to live long enough for the X-ray transition to take place and since it can be destroyed by a collision with a nearby atom there is a competition between the transition time and the collision time. The probability of X-ray emission thus depends strongly on the density of the gas.

The first experimental evidence for quasi-molecular X rays was obtained by Saris *et al.* (*Phys. Rev. Lett.*, **28**, 717; 1972) who bombarded argon-saturated silicon targets with 70–600 keV argon ions. This gave a continuous X-ray distribution between the characteristic L and K X-ray lines of argon. These X rays are attributed to transitions to the $2p\pi$ level of the molecular system. In a later experiment they confirmed the density dependence of the X rays and compared the results from various targets. This work has since been extended and confirmed by several other groups.

The X rays from the quasi-superheavy nucleus with $Z=132$ have been found by Mokler *et al.* (*Phys. Rev. Lett.*, **29**, 827; 1972). They bombarded a gold target with iodine and some of

their X-ray spectra are shown in the figure. The broad peak at about 8 keV is attributed to transitions in the quasi-superheavy nucleus. This occurs at the expected energy and detailed calculations of other possible processes showed that none of them could account for this peak. Similar results were obtained with other targets.

It is now possible to make relativistic many-electron calculations of the energies of the states during the collision process and hence to find the energies and intensities of the expected X rays

for different interacting ions as a function of energy. The detailed experimental confirmation of these results shows that the phenomenon is essentially understood. There still remain many questions that require further study, in particular the possibility of other mechanisms contributing to the X-ray emission and the angular distribution of the emitted radiation. A review of the present status of this work has recently been published by Armbruster (*Enrico Fermi Summer Course*, LXii; 1976). □

Muscle disease at Durango

from Jeremy Brockes

The Fifth International Scientific Conference sponsored by the Muscular Dystrophy Association of America, entitled Pathogenesis of the Human Muscular Dystrophies, was held at Tamarron, Durango, Colorado from June 21 to 25, 1976. The program Coordinator was Dr Lewis P. Rowland of the Columbia-Presbyterian Medical Center, New York.

THE human muscular dystrophies are a complex of hereditary disorders whose underlying mechanisms are completely unknown. It was thus appropriate that the seventy contributors to the Fifth MDA conference considered many aspects of normal and abnormal muscle biology. In this report, I have concentrated on new information that may be of direct relevance for understanding or diagnosing these diseases.

In terms of understanding the mechanism of a muscle disease, the most exciting presentations came on the first day when recent work on the autoimmune basis of myasthenia gravis was presented by J. Lindstrom (Salk Institute) and D. B. Drachman (Johns Hopkins University). It has recently been shown in a number of laboratories that the serum of myasthenia patients contains antibodies that react with acetylcholine receptor. Drachman showed that certain features of the disease can be passively transferred to a mouse with purified gamma globulin from a patient. The mouse exhibits decremental responses to nerve stimulation together with a decrease in endplate acetylcholine receptors and other characteristic features of the myasthenia syndrome. These effects are in part dependent on the third component of complement, as evidenced by the ability of cobra venom factor (which depletes C3) to abrogate the transferred disease, but independent of later components as

shown by transfer into the C5 deficient strain. The effects of antibody were further considered by Lindstrom in an animal model. When purified electric eel receptor is injected into rats, the animals mount an immune response which leads to the syndrome of Experimental Autoimmune Myasthenia Gravis (EAMG). In the acute phase of this disease, approximately 10–15 days after injection, cross-reacting antibodies attach to junctional receptors in the synaptic cleft and attract phagocytic cells, mainly macrophages. This leads to a partial disjunction of nerve and muscle, and further morphological abnormalities in the post-synaptic membrane which were described by A. G. Engel (The Mayo Clinic). The acute phase is succeeded about two weeks later by a chronic reaction in which antibody-induced modulation of junctional receptors leads to failure of junctional transmission, progressive weakness and (usually) death. The reason for the apparent breakdown of tolerance to nicotinic acetylcholine receptors in the human disease, and the source of antigen that drives the response, are not known. In view of the close connections between myasthenia and the thymus, one candidate for the responsible antigen is the thymic myoid cell. Drachman reported that cultured rodent myoid cells possess acetylcholine receptors on their surface as evidenced by α -bungarotoxin autoradiography and acetylcholine iontophoresis.

In contrast to myasthenia, the human muscular dystrophies present a confusing picture. Presentations by T. Munsat (University of Southern California) and P. Becker (University of Göttingen) attested to the heterogeneity of these disorders, and the problems of classifying them. Most of the investigations on human muscular dystrophy were on the Duchenne type, and several investigators spoke of the problems of obtaining adequate biopsy material. A

variety of biochemical, physiological and anatomical abnormalities were described. While there was some talk of an underlying membrane defect, the evidence for this is not yet compelling. Controversies about the neurogenic or myogenic origin of dystrophy continue unabated, with disagreements about counting motor units and interpreting physiological recordings. The interpretation of transplantation or culture experiments in experimental animals with muscular dystrophy is complicated by possibilities of early inductive abnormalities between ectoderm and mesoderm (suggested by A. McComas (McMaster University)), and the failure of dystrophic muscle to retain some of its characteristics in culture.

One approach to muscular dystrophy is to regard it as resulting from a basic defect expressed in all cells, but of particular consequence for muscle. A. D. Roses and S. Appel (Duke University), reported their studies of abnormal phosphorylation of components in or under the erythrocyte membrane. When incubated with labelled ATP, erythrocytes from patients with Duchenne dystrophy show a significant increase in spectrin phosphorylation over controls. If the phosphorylated spectrin is analysed by isoelectric focusing, a subset revealed by this method shows a clear difference from controls. While the molecular basis of this difference is not yet clear, it is not shown by the mothers of dystrophics, and therefore presents an exciting possibility for prenatal diagnosis. In the case of patients with myotonic dystrophy, Appel reported a decrease in the phosphorylation of erythrocyte band 3, a major transmembrane glycoprotein in human erythrocytes thought to be responsible for ion transport. Such erythrocytes also show a defect of Ca^{2+} -dependent K efflux in certain circumstances, and the defect in phosphorylation could be related to decreased Ca^{2+} entry or a failure to activate the K^+ conductance. The relationship of the erythrocyte abnormalities to those in the muscle cell is unclear. In a lucid presentation, R. Layzer (University of California, San Francisco) pointed out that the same hereditary defect, for example in phosphofructokinase isozymes, can have quite different consequences in the two cell types. Nonetheless, the erythrocyte work is an encouraging new direction, particularly for diagnosis.

Meanwhile it is possible that less direct approaches to therapy may prove useful. The importance of the balance between rates of protein synthesis and degradation was stressed for general metabolism by A. L. Goldberg (Harvard Medical School), and for the regulation of acetylcholine receptor levels by D. M. Fambrough (Carnegie

Institute). A. Stracher (State University of New York) discussed the properties of low molecular weight, non-toxic, protease inhibitors such as pepstatin and presented interesting preliminary evidence of their effects in retarding degeneration of dystrophic chick muscle explants and monolayers. The presence of segmental necrosis in Duchenne muscle fibres was stressed by several speakers, and J. Desmedt (University of Brussels) presented physiological evidence for collateral reinnervation of the resulting aneural segments. It might be possible to promote controlled regeneration in dystrophic muscle, with recovery of function after reinnervation.

While there are new diagnostic possibilities, the basic defects in dystrophic muscle remain as elusive as those in cancer cells. Nonetheless, the progress in myasthenia illustrates how advances in basic research can quickly be important in a clinical context. Thus the breadth of the MDA's support, and their personal involvement as exemplified in an address by Jerry Lewis, the National Chairman, offer cause for hope. □

Protein folding experiments: do proteins cheat?

from Barry Robson

THOSE fundamental components of biological machinery, the proteins, are synthesised by the ribosomes of living cells as linear polymers of twenty different kinds of amino acid. Investigators who seek to explain, and even predict, the functional three-dimensional structures of protein molecules on the basis of their amino acid sequences alone are often criticised for neglecting the presence of the ribosome and other biological structures when the protein folds up. Their defence has been that proteins can be unfolded by certain solvents so that a fully unfolded, flexible and random structure is attained, and then refolded without the aid of the ribosome or anything more complex than water, salts, and a healthy temperature and pH. Proving experimentally that the amino acid sequence carries all the information for the three-dimensional biological structure is rather like examining a student, however, for in order to prove that the student knows his stuff it is important to ensure that he can think out the problem set to him without taking any written record into the

examination room. The experiments of Garel, Nall, and Baldwin (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 1853; 1976) raise the possibility that some protein molecules at least may cheat because they cannot be fully unfolded but retain some elements of their original three-dimensional structure before refolding.

Baldwin's group unfolded the protein ribonuclease A in aqueous solutions of guanidine hydrochloride or urea, and carefully analysed the kinetics of refolding when the guanidine hydrochloride or urea was diluted out. They conclude that the unfolded protein is an equilibrium mixture of two species, one which slowly refolds to the biologically functional, compact state and one which refolds to the same state but 450 times more rapidly. The protein was described as fully unfolded on the basis of difference spectroscopy techniques, which measure the exposure of aromatic amino acid residues to the solvent. Although there is another instance of complete unfolding in terms of solvent exposure, but not in terms of other criteria (Robson and Pain, *Biochem. J.*, **155**, 331-334; 1976) the fraction of rapidly refolding form (about one fifth) is remarkably constant over a variety of conditions. The report of Baldwin's group is thus a cautionary tale: proteins may sometimes cheat because of the invigilance, perhaps unavoidable, of the investigators. Although four-fifths of the unfolded ribonuclease A molecules presumably do not cheat and eventually also reach the correctly folded state, apparently by initial conversion to the rapidly re-folding form (Hagerman and Baldwin, *Biochemistry*, **15**, 1462-1473; 1976), it is possible that there are many proteins with an even more stable rapid refolding form which constitutes a very much larger fraction of the unfolded population. Such proteins would have no option but to cheat, unless some more thorough method or combination of methods of denaturation were used.

Experiments on other apparently completely unfolded proteins have frequently demonstrated complex refolding kinetics, but have not necessarily implied the presence of a stable unfolded and fast refolding form. The relatively unstable intermediates detected in this way are generally believed to represent partly ordered structures initiating a direct and readily traversable route to the folded state, and so guide the folding process. Such guidance or "nucleation" is in fact theoretically necessary in order to account for the rapidity of folding processes which are many orders of magnitude faster than would be predicted from a purely random search of all possible polymer configurations. The

question therefore arises as to whether the fast folding species of ribonuclease A is a partly ordered nucleating structure in the usual sense, which just happens to have a high degree of stability.

The authors note, however, that there is an alternative hypothesis based on the proposal of Brandts, Halvorson, and Brennan (*Biochemistry*, **14**, 4953-4963; 1975) that the amino acid residue proline may undergo *cis-trans* interconversion in the unfolded form of a protein. The fast refolding species could therefore represent forms in which all proline residues are in the correct configuration as found in the correctly folded protein. Although purists might argue that such a form would be a "nucleating structure" in the most general sense, the term usually refers to rapidly formed chain structures in which at least several amino acid residues have a fairly specific position in space relative to one another. It would therefore be rather unfair to accuse the protein molecule of cheating if the only structure it retained were the correct configuration of the proline groups.

Even the most detailed test-tube experiments on the configurational statistics of unfolded proteins may not be able to rule out the possibility of small elements of local structure important to the refolding process. Perhaps the real proof that proteins carry all the information for their own folding resides in computer simulations in which the investigator has full control over the initial conformation of the protein molecule. □

NASA Goddard gamma-ray symposium

from F. W. Stecker and C. E. Fichtel

An international symposium on The Structure and Content of the Galaxy and Galactic Gamma Rays was held at NASA Goddard Space Flight Center in Greenbelt, Maryland on June 2-4, 1976.

THE emphasis of the conference was placed on the relationship of γ -ray astrophysics to other fields of galactic astronomy.

D. Thompson and R. Hartman (Goddard Space Flight Center) presented the results from the Second Small Astronomy Satellite, SAS-2. Evidence for pulsed γ radiation from

four radio pulsars, PSR 0531+21, PSR 0833-45, PSR 1818-04 and PSR 1747-46 was reported. Only one radio pulsar has been seen at optical and X-ray wavelengths. In addition, several general features in the γ -ray data were correlated with galactic structural features. The γ rays seen by SAS-2 from the direction of Cygnus were determined to have a periodicity of 4.8 h, matching the frequency and phase of the X-ray source Cygnus X-3.

A description of the COS-B satellite γ -ray detector was given by B. Swanenburg (Huygens Laboratorium). Preliminary results were presented by J. Paul (Saclay), H. Mayer-Hasselwander (Max-Planck Institute, Garching) and R. Buccheri (University of Palermo). These results included confirmation of a strong γ -ray source near galactic coordinates $195^\circ, +5^\circ$, and an indication of a change in the intensity of the Vela source since the time of the SAS-2 observations. The temporal analysis of PSR 0833-45 indicated that the γ -ray pulses were quite narrow.

G. Share (US Naval Research Laboratory) noted that the galactic γ -ray energy spectrum below 30 MeV showed the predicted steep shape characteristic of bremsstrahlung radiation. J. Grindlay (Center for Astrophysics, Cambridge, Massachusetts) noted that there are now two known sources emitting γ rays at energies $\geq 10^{12}$ eV.

P. Sturrock (Stanford University) suggested that a cascade process resulting from electron-positron pair creation in the pulsar magnetic field may be the cause of the pulsed γ radiation. H. Ogelman (Middle East University, Ankara) pointed out that pulsars radiate predominantly in the 1–1,000 MeV range. W. B. Burton (National Radio Astronomy Observatory) and N. Scoville (University of Massachusetts), both presented new observations of the 2.6 mm CO emission line which indicated that molecular clouds in the Galaxy (mostly H_2) are concentrated between galactic radii of 4 and 8 kpc in contrast to the atomic hydrogen, which is relatively uniform in distribution at radii from 4 to 14 kpc. The measurements also show that H_2 clouds are more tightly confined to the galactic plane than atomic hydrogen gas. E. Jenkins (Princeton University) noted that ultraviolet measurements of the molecular hydrogen in the local region of the galaxy give a value of the density a factor of four lower than that implied from the CO observations for a galactic radius of 10 kpc, although the results are not necessarily in conflict owing to selection effects.

W. Roberts (University of Virginia) discussed the spiral density wave theory of galactic structure and stated that the apparently striking separation of

atomic and molecular hydrogen could be the result of stronger compression of gas in the inner galaxy, and an increase of the frequency at which the interstellar gas is periodically compressed.

J. Baldwin (Cavendish Laboratory, Cambridge) noted that the non-thermal galactic radiation implies a much broader distribution of the cosmic ray electrons than of matter in the galaxy. This has been found also to be the case in other spiral galaxies. J. Seiradakis (Max-Planck-Institut, Bonn) showed that, within the present limited statistics, the radial distribution of pulsars is generally correlated with that of other young (Population I) galactic objects. Both G. Fazio (Center for Astrophysics) and J. L. Puget (Observatory Meudon) discussed the significance of the local molecular hydrogen clouds in terms of infrared and γ -ray emissions. Puget concluded that the γ -ray observations indicate that the cosmic ray densities inside and outside clouds are similar.

R. Lingenfelter (University of California, Los Angeles) discussed the γ -ray lines emitted by the excited nuclei produced by interactions of cosmic rays with interstellar matter. He predicted that the 4.4 MeV carbon line would be the most intense. D. Arnett (University of Illinois) described the possibility of studying the results of nucleosynthesis in supernovae by examining the γ -ray lines emitted by the end products, and noted that the time interval after the explosion before the supernova envelope becomes transparent to γ rays was much longer than had been estimated earlier.

E. Parker (University of Chicago) gave a comprehensive lecture on cosmic ray propagation and galactic containment, in which he noted that the cosmic rays, the magnetic field, and the interstellar gas strongly affect each other dynamically. He concluded that if cosmic rays leave the galaxy, it must be as a consequence of collective cosmic-ray pressure.

The part that γ -ray astronomy can play in determining the large-scale galactic structure was then discussed. D. Kniffen (Goddard) emphasised that the cosmic ray electrons and protons can be studied independently by looking at different portions of the γ -ray spectrum, and that γ -ray observations should be particularly valuable in searching for spiral structure. F. Stecker (Goddard) showed that galactic γ -ray emission is correlated with the molecular cloud distribution in the galaxy and that the deduced cosmic ray distribution appears to follow the supernova remnant and pulsar distributions in the galaxy, favouring a galactic supernova origin for most

cosmic rays. He also discussed γ -ray implications for cosmic ray propagation and the galactic contribution to the high latitude γ -ray background.

After almost two decades of strenuous effort developing instruments of sufficient sophistication and sensitivity, there have been significant advancements in γ -ray astronomy in the past few years. Not only has the general level of the galactic radiation been established, but also its broad features. With improved angular resolution and much greater sensitivity, γ -ray astronomy together with astronomy at other wavelengths hold great promise for the study of our Galaxy, other galaxies and high energy processes in astronomical objects. $\square$



A hundred years ago

A FALL of meteorites, we learn from *Aftonblad*, took place on June 28, between 11 and 12 a.m., near Ståldalen, a station on the Swedish Central Railway, in the northernmost part of Örebro län. Several fell, some on the ground and others in a lake. Two were found, one about the size of the fist and weighing $4\frac{1}{2}$ lbs., the other smaller. Eye-witnesses stated that a loud whistling was first heard in the air from west to east, and a light was plainly distinguishable; although the sky was clear and cloudless, thereafter two very sharp reports were heard, the second succeeding the first after a momentary interval, followed by several others less sharp, resembling thunder, after which the falling stones were observed by eight or ten persons; and finally, there was seen in the air a whirling smoke, not very high up. A meteor was observed simultaneously at Stockholm and at other places. At thirteen English miles south-west of Linköping it was seen first in a north-westerly direction pretty high up in the sky, and it then sank down in about ten seconds towards the horizon in the west. It had the appearance of a large pear a foot long, which, notwithstanding the bright sunshine, left behind a clear shining streak of six or eight feet in apparent length, which finally broke up into a multitude of starlike sparks. Here no noise was heard. According to a communication from the Stockholm Meteorological Bureau, there is reason to believe that the phenomena arose from the "kulblix" (*foudre globulaire*), which generally appears as a luminous round object, and often, on approaching the ground, assumes a lengthened form and a blinding white colour, and bursts asunder, commonly with a loud report. from *Nature*, 14, August 3, 300; 1876.

review article

Fundamental research in molecular biology: relevance to medicine*

M. F. Perutz†

Molecular biology has given us new insights into many phenomena of clinical importance; it has suggested new therapeutic approaches to several diseases and has provided sensitive new methods for detecting chemical carcinogens. None of these applications had been foreseen.

MOLECULAR biology is sometimes said to be of little medical significance because it has not cured anyone yet. A hundred years ago the same might have been said of histology. At that time cellular anatomy and pathology began to improve our understanding of many diseases; today molecular anatomy and pathology give us much deeper insights. I shall illustrate this theme by examples taken from four different fields of research. The first two are from the fields with which I am most familiar, the structure and function of proteins; the second two are from molecular genetics. I shall show first how research on the structure of protein-digesting enzymes has led to an understanding of blood clotting and other important functions whose relationship to digestion no one had suspected. I shall then relate how my own work on the structure of haemoglobin has improved our understanding of genetic diseases and how basic research on autoxidation of haemoglobin has suggested a way of treating certain inherited anaemias. My next two themes are concerned with the effects of ultraviolet light and chemical carcinogens on nucleic acids and their relationship to human cancer. Finally, I shall ask whether any of this research could have been planned from the outset with a view to its medical applications.

Proteolytic enzymes and their inhibitors

In the 1950s the structure and catalytic mechanism of enzymes still ranked as one of the great unsolved problems in biochemistry. At that time two of my colleagues, B. S. Hartley and D. M. Blow, became interested in the proteolytic enzyme chymotrypsin, simply because they regarded it as the most hopeful and interesting one to attack. This enzyme is closely related to two other pancreatic proteolytic enzymes, trypsin and elastase. Chymotrypsin splits fastest those peptide bonds that follow amino acids with aromatic side chains, while trypsin goes for peptide bonds that follow basic side chains, and elastase for those with very short side chains. All three enzymes are secreted into the jejunum in the form of inactive precursors, known as chymotrypsinogen, trypsinogen and proelastase; all three precursors are activated by trypsin.

The pancreas also makes a small protein which scavenges and inhibits any traces of free activated trypsin. This is the pancreatic trypsin inhibitor.

Hartley and his colleagues determined the amino acid sequence of chymotrypsin and chymotrypsinogen¹ while Blow and others solved the three-dimensional structure of chymotrypsin by X-ray analysis². Other workers followed with the structure of chymotrypsinogen, of trypsin, of elastase, of the trypsin inhibitor and of the trypsin-trypsin inhibitor complex. Together, Blow, Hartley and their colleagues worked out the mechanism of activation and catalysis³, and R. Huber and Blow that of inhibition⁴.

Chymotrypsin consists of 242 amino acid residues in three separate chains, which are linked by disulphide bonds. Its active site contains a serine, a histidine and an aspartate juxtaposed so that protons can shuttle backwards and forwards between them, forming what Hartley and Blow called a charge relay system.

The substrate is recognised by a pocket that lies next to the active site and is so tailored that it attracts the side chain of the amino acid preceding the peptide bond to be split. In the inactive precursor that specificity pocket is closed. Trypsin activates the precursors by splitting their chains in a way that allows the pocket to open. The pancreatic trypsin inhibitor mimics a substrate by presenting to the enzyme a side chain which its specificity pocket recognises, but the peptide bond following that side chain is so shielded from water that the enzyme cannot split it. Instead, the inhibitor remains firmly attached to the enzyme in a position resembling the one fleetingly occupied by a substrate at the first intermediate stage of catalysis⁵.

The mechanisms of activation, recognition and catalysis of chymotrypsin first became known in outline in 1969, and the way in which the pancreatic trypsin inhibitor blocks trypsin in 1972; some further important details have been added since. Whenever nature has invented an effective and versatile device, it tends to exploit it over and over again for different functions. The same mechanisms of activation, recognition, catalysis and inhibition have now been found in at least four other functions: the clotting of blood, the dissolution of blood clots, the disposal of complexes of antigen and antibody by complement, and the lowering of blood pressure by the release of kinins. The raising of blood pressure by the release of angiotensins is also due to a proteolytic enzyme known as renin, but this is an acid proteinase related to pepsin, and its structure is still unknown. The interplay of protein digesting enzymes and their inhibitors seems to be important also in degenerative

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diseases such as pulmonary emphysema and rheumatoid arthritis.

Suitable stimuli to blood platelets set off a chain reaction which causes blood to clot with very high velocity. This is due to a cascade of proteolytic enzymes and auxiliary proteins whose purpose it is to activate thrombin and liberate it from the platelets into the plasma (Fig. 1). It is now certain that part of the thrombin molecule has a structure which is almost identical to that of trypsin; in the clotting factors XIa, IXa and Xa, the parts of the sequences that have so far been analysed, are also like those of trypsin. The precursor of each of these enzymes is activated by the preceding enzyme in the clotting cascade by the same kind of mechanism by which trypsin opens the specificity pocket in trypsinogen or chymotrypsinogen, and each possesses the same charge relay system for the hydrolysis of peptide bonds. The functioning of the cascade depends on the presence of calcium ions and on nutrition with adequate amounts of vitamin K.

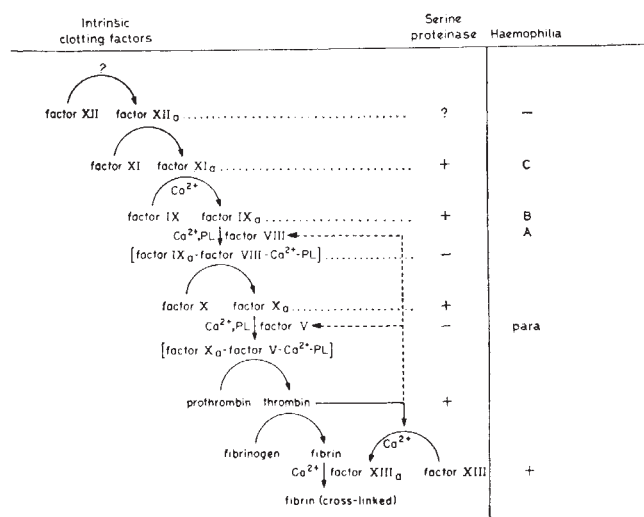


Fig. 1 Cascade of intrinsic clotting factors. Those marked + are enzymes similar to trypsin (reproduced, with permission, from the article by Davie and Kirby⁶).

The clotting factors possess several refinements which the pancreatic enzymes lack. Their specificities are probably greater and they are activated not in free solution, where they would be too widely dispersed, but on the surface of phospholipid membranes of blood platelets where they are crowded together. Prothrombin, the precursor of thrombin, has an extra 274 amino acids which are not found in trypsinogen, forming an attachment which binds it to the membrane, but only in the presence of calcium ions. On activation by factor Xa this attachment is split off and thrombin is released into the plasma. A great advance has recently been made in our understanding of the role of calcium and its dependence on vitamin K. It has been found that calcium ions are bound to prothrombin by 10 residues of a new amino acid, γ -carboxyglutamate; the residues lie near the amino terminus of the chain. The new amino acid is made after the polypeptide chain of prothrombin has been assembled in the liver, by an enzyme system that adds a second carboxyl group to each of the first ten glutamates; and this subsidiary enzyme system that adds carboxyl groups requires vitamin K as a cofactor. In the clotting cascade, the other three trypsin-like factors or enzymes also require calcium in order to be activated, which implies that they are subject to the same vitamin K dependent modifications as prothrombin. These discoveries explain how vitamin K antagonists interfere with blood clotting: by reducing the calcium affinity of prothrombin and of three

other clotting factors they prevent their binding to the phospholipid membrane and thereby inhibit their activation⁷.

The therapeutic uses of vitamin K antagonists were discovered long before its mode of action was understood, but the new discoveries will allow the antagonists to be applied more rationally and with greater knowledge of their possible side effects. For example, the presence of the new amino acid γ -carboxyglutamate in prothrombin suggested that it might be worth while to search for it in other calcium binding proteins. This search has led to the isolation of the amino acid from a protein in the bones of 14-week-old chicken, which suggests that it may be needed for the deposition of calcium in developing bones⁸, and explains why treatment of women during the first 3 months of pregnancy with anticoagulants of the dicoumarol type has led to bone anomalies in the foetus. Quite recently, γ -carboxyglutamate has also been found in a small protein isolated from adult bones of several species, including humans. The amino acid forms part of a peptide whose sequence bears no resemblance to that of the N-terminal peptide of prothrombin, showing that it is part of a quite different protein specific to bone, and indicating that vitamin K is essential for the formation and maintenance of bone structure³⁷.

There are at least two natural anticoagulants which prevent clotting getting out of hand. An excess of free thrombin in the plasma stops activation of more prothrombin by splitting off its first 156 residues, thus detaching it from the phospholipid membrane before it can be activated. A plasma inhibitor, AIII antithrombin, inhibits thrombin and other clotting factors by a mechanism apparently similar to that by which the pancreatic inhibitor blocks trypsin, except that by itself AIII antithrombin acts much more slowly. It has recently been found that heparin exerts its instant anticlotting effect by accelerating the reaction of AIII antithrombin with thrombin and with other factors. Patients with a congenital deficiency of AIII antithrombin have an increased tendency to thrombosis⁹, which shows that this inhibitor is an essential part of the checks and balances in the clotting system.

AIII antithrombin is one of six different antiproteases so far discovered in human plasma. Another of the six that has recently assumed medical importance is α_1 -antitrypsin, because people with an inherited deficiency of this inhibitor are predisposed to pulmonary emphysema. The mechanism of lung destruction in these patients is still unclear but is believed to be due to digesting enzymes from granulocytes and macrophages. These may cause emphysema even in people with normal levels of α_1 -antitrypsin if they are released in excess by chronic lung irritation. It now looks as if pulmonary emphysema may be only one example of a degenerative disease where protein-digesting enzymes have a decisive role. For example, one such enzyme, though of a type different from trypsin, has been found in the joints of rheumatoid arthritis patients. Knowledge of the three-dimensional structure of these enzymes may open the way to the synthesis of tailor-made inhibitors for the treatment of such diseases.

Haemoglobin diseases

In 1949 Pauling, Itano, Singer and Wells published a paper entitled "Sickle Cell Anaemia, a Molecular Disease"¹⁰. They showed that homozygotes suffering from this autosomal recessive disease had a haemoglobin which differed from the normal by the absence of two negative charges. Ingram later discovered that this was due to the replacement of a single amino acid residue among the 287 in the half molecule of haemoglobin; one glutamate was replaced by a valine. (Recently the same replacement of a single glutamic acid by a valine has been found in a variant human α_1 -antitrypsin(S). The replacement is harmless in heterozygotes (SM) carrying both the normal (M) and the variant (S) gene, but it may predispose SS homozygotes to

pulmonary emphysema¹¹). This was the first time that a genetic mutation was shown to cause the replacement of an amino acid in a protein. A search for other haemoglobin diseases soon followed, and now more than 200 different abnormal haemoglobins are known, which give rise to a variety of haematological disorders whose cause had been unrecognised before; these may affect both the synthesis and life span of red blood cells.

The dual respiratory function of haemoglobin depends on a reversible change of structure. The venous form of

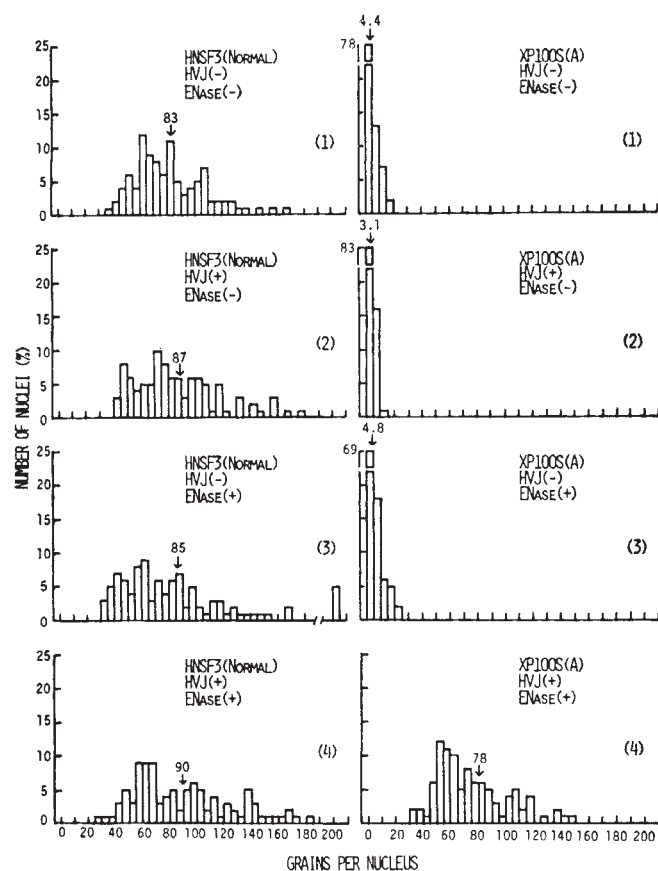


Fig. 2 Histograms showing numbers of silver grains produced by the disintegration of ^3H labels after ultraviolet irradiation in nuclei of normal skin fibroblasts (left) and fibroblasts from a xeroderma pigmentosum patient (right). HNSF3: normal skin fibroblasts. XP100S(A): xeroderma pigmentosa cells group A. HVJ: ultraviolet inactivated Sendai virus. ENase: endonuclease from T4 infected *E. coli*. (Reproduced, with permission, from Tanaka *et al.*²¹.)

haemoglobin has a low affinity for oxygen and a high one for hydrogen ions, CO_2 and 2,3-diphosphoglycerate; in the arterial form these relative affinities are reversed. Efficient respiratory transport depends on correct regulation of the equilibrium between the two forms. My colleagues and I have determined their three-dimensional structure in atomic detail^{12,13}. H. Lehmann and I then attempted to interpret the clinical symptoms of the haemoglobin diseases in stereochemical terms¹⁴. In most of them the abnormality consists of the replacement of a single amino acid residue, as it does in sickle cell anaemia, but the position of that residue is crucial in deciding the nature of the symptoms. If the residue lies on the surface, as often happens, there may not be any symptoms, at least in heterozygotes, because the haemoglobin molecule hardly "feels" the wrong residue.

In homozygotes, on the other hand, superficial replacements may reduce the solubility of the haemoglobin in the red cell. This happens in sickle cell anaemia where the venous form of haemoglobin actually crystallises out. Prevention of this crystallisation would relieve the disease, but has proved very difficult. The structure of the areas of contact between neighbouring molecules of sickle cell haemoglobin is now being worked out in atomic detail and may form the basis for a synthetic approach to the inhibition of crystallisation.

The surface of the haemoglobin molecule is studded with electrically charged amino acid side chains which ensure its high solubility in water. Its interior structure, on the other hand, is stabilised by hydrocarbon side chains which make it impenetrable to water. In several abnormal haemoglobins this water-repellent armour is breached, so that they become unstable and form amorphous precipitates in the red cell known as Heinz bodies. Similar denatured precipitates are seen in thalassaemia where the red cells contain unequal numbers of α and β chains. The precipitates make the red cells more rigid and therefore more fragile, and this was believed to be the main cause of the associated haemolytic anaemias. Denaturation of haemoglobin is also accompanied by oxidation of the haem iron from its normal ferrous to the ferric state. It has now been found that it is this oxidation rather than the presence of denatured haemoglobin precipitates which mainly causes haemolysis. The mechanism is this. In the first step of the reaction the iron-bound oxygen molecule is reduced to superoxide ion which may then dissociate from the haem and produce hydrogen peroxide ion, hydroxyl radicals and singlet oxygen in the surrounding water¹⁵. These are all oxidising agents, hydroxyl radical being the most powerful among them, and they attack the lipids of the cell membrane with resulting haemolysis. The obvious remedy is the administration of drugs designed to trap free radicals such as vitamin E or 2,3-dihydrobenzoic acid. These are now under trial^{16,17}. In the long term practical results of much wider importance should result from the study of the abnormal haemoglobins. In the first place they have shown that the genetic code in humans is the same as in *E. coli*, which is a prerequisite for relieving congenital diseases by any kind of genetic engineering. They have proved that human genes are subject to a fairly high rate of mutation (the frequency of abnormal haemoglobins in the human population may be greater than 1 in 500), but that these mutations may be harmless if they merely replace residues at the surface of enzymes away from their active sites. Interior replacements on the other hand, may affect the function and stability of enzymes. In favourable circumstances knowledge of their three-dimensional structure may allow us to mitigate such effects.

I started to study haemoglobin by X-ray crystallography in 1937 because at that time the structure of proteins seemed the most important unsolved problem in biochemistry, but I never dreamt that its solution would one day throw light on the nature of inherited diseases.

Xeroderma pigmentosum

The mutations which give rise to the abnormal haemoglobins occur spontaneously, perhaps by random errors inherent in the genetic process. It had long been known that the rate of mutation can be enhanced by external factors such as radiation and certain chemicals. It had also been suspected that some of the same factors which produce mutations give rise to cancer, but no exact correlation had been shown.

Ultraviolet light damages DNA by causing two pyrimidine bases, usually, but not always, on the same strand, to become covalently linked. In ultraviolet-sensitive bacteria this linkage inhibits DNA replication and thereby stops

further growth. But there are strains of the same bacteria which are resistant to ultraviolet and it turns out that these possess an enzyme system which excises the dimers and repairs the gap, thus allowing growth to start again¹⁸. Now it appears that normal human skin cells have an enzyme system for the repair of ultraviolet damage similar to that of ultraviolet resistant bacteria. There is a rare autosomal recessive human disease in which the skin is extremely sensitive to ultraviolet light; it is called xeroderma pigmentosum. Sufferers from this disease—the homozygotes—are also very liable to skin cancer. In 1969 J. E. Cleaver discovered that fibroblasts from such patients lack an enzyme necessary for the repair of ultraviolet-damaged DNA¹⁹. Investigators later found that there are six genetically distinct types of enzymatic deficiency, that is six complementation groups, five of which are deficient in DNA repair and the sixth in another as yet unknown function²⁰; Japanese workers have now shown that ultraviolet resistance can

histidine is scarce and switching it off when it is plentiful. Certain mutant strains of *Salmonella* cannot grow unless histidine is supplied in the medium; this has turned out to be due to mutations in the histidine genes. Ames selected four such strains. In one of them the DNA differs from that of the wild type by the replacement of a GC by an AT pair, or vice versa. In the remaining three strains the DNA carried mutations of a kind first correctly interpreted by Crick, Barnett, Brenner and Watts-Tobin when they established the nature of the genetic code^{22,23}. Decoding of the sequence of nucleotide bases makes sense only if the reading frame is positioned correctly with respect to the succession of coding triplets. Certain mutagens such as proflavine intercalate between the base pairs of DNA and thereby interfere with its replication so that base pairs are either added or deleted, and the reading frame is thus shifted.

Three of the four strains of *Salmonella* used by Ames

Table 1 Correlation between carcinogenic and mutagenic activity of chemicals

	car + mut +	car 0 mut 0	car + mut 0	car 0 mut +	?	correlation (%)
Aromatic amines and so on	21	9	2	—	12	94
Fungal toxins and antibiotics	8	2	—	2	2	83
Esters, epoxides, carbamates and so on	14	6	4	2	2	77
Nitro aromatics and heterocycles	26	1	—	3	2	90
Miscellaneous heterocycles	1	7	3	—	—	73
Miscellaneous nitrogen compounds	7	2	2	2	—	69
Nitrosamines and so on	20	2	1	—	—	96
Polycyclic aromatics	27	7	—	3	1	92
Miscellaneous aliphatics and aromatics	1	10	4	—	5	73
Alkyl halides	16	1	3	2	2	77
Azo dyes and diazo compounds	12	2	—	1	2	93
Cigarette smoke condensate	1	—	—	—	—	100
Common biochemicals	—	42	—	—	—	100
Totals	154	91	19	15	28	88

106 non-carcinogens 14% positive, but
86% negative

173 carcinogens 89% positive, but
11% negative

be restored to all those five types of fibroblasts that are deficient in DNA repair by an enzyme which is an endonuclease from T4 phage infected *E. coli*²¹ (Fig. 2). Their results are exciting because they suggest that it may become possible to treat patients with this kind of enzyme, even if it is not yet clear how it could be applied. Quite recently, an altered endonuclease concerned with the repair of depurinated sites has been found in fibroblasts from two of the complementation groups that were known to be deficient in DNA repair²⁸.

Detection of chemical carcinogens

Discovery of the cause of xeroderma pigmentosum was important because it linked the occurrence of a cancer with a mutagenic event whose nature and repair could be explained in the same molecular terms as in bacteria. Evidence in favour of a causal link between the frequency of somatic mutation and the incidence of cancer has long been accumulating, yet many of the most potent chemical carcinogens seemed to have no mutagenic effect in micro-organisms. This paradox was finally resolved by Bruce Ames. Ames had devoted nearly 20 years to the study of the control of protein synthesis in *Salmonella typhimurium*. His work, like that of Hartley and Blow on chymotrypsin and mine on haemoglobin, was undertaken without any foreknowledge of possible applications.

The chromosome of *Salmonella* contains an operon that codes for histidine biosynthesis. Transcription of the enzymatic genes of this operon into messenger RNA is controlled by an operator gene which responds to the level of histidine in the cell, switching transcription on when

carry mutations of this type in the histidine genes^{24,25}. Ames plated these histidine-requiring strains of *Salmonella* on a histidine-free nutrient, applied chemical carcinogens and waited for the growth of colonies in which a reversion of the original mutation had allowed the resumption of histidine biosynthesis. The presence of 10⁸ organisms on each plate allowed rare genetic events to be detected. The smallness of the bacterial chromosomes increased the likelihood of reversions of the original mutation to other mutations which might be lethal. The time needed for one experiment was only 2 d.

Initially only some of the known chemical carcinogens caused growth of revertant colonies but Ames and his colleagues improved the sensitivity of his system step by step until he was able to prove a strong correlation between carcinogenicity and reversion. He introduced two additional mutations: one raises the sensitivity by eliminating the repair mechanism already mentioned, that repairs ultraviolet and other damage, and the other strips the bacterial surface of part of its protective lipopolysaccharide layer which makes it more permeable to large molecules. He also increased mutagenesis in his tester strains by incorporating a plasmid²⁶ which appears to channel DNA damage down an error-prone repair pathway (SOS repair)²⁷⁻³⁰.

Ames made one more improvement in the system which proved decisive. It had been discovered that in mammals many compounds are non-carcinogenic as such, but are converted into carcinogens by hydroxylating enzymes in the liver. Ames decided to mimic this conversion by adding rat or human liver homogenate together with a system for generating nicotinamide adenine nucleotide diphosphate

to his cultures³¹⁻³³. The results show a strong correlation between carcinogenesis in mammals and mutagenesis in *Salmonella*^{34,35}. Ninety per cent (157/175) of the carcinogens were mutagenic in the test including almost all of the known human carcinogens that were tested. Despite the severe limitations inherent in defining non-carcinogenicity, few "non-carcinogens" showed any degree of mutagenicity (Table 1). The mutagens include tar from cigarette smoke and oxidative hair dyes (Fig. 3)³⁶. Another mutagen is chloroacetaldehyde, a possible metabolite of dichloroethane and of vinylchloride, two chemicals of which several million tons are produced in the USA each year. In the first instance, all these mutagens probably act by stopping DNA replication and thereby inducing the error prone SOS repair mechanism. Mutagens which do not do this, such as EMS, base analogs and deaminating agents, appear to be weakly or not carcinogenic (M. Radman, private communication). Study of the SOS repair mechanism would therefore seem a promising approach to the understanding of radiation and chemically induced carcinogenesis.

Ames' method offers a cheap, quick and sensitive way of screening chemicals for mutagenicity. For instance, 1 ng of 2-aminoanthracene doubled the spontaneous reversion rate of about 20 colonies, and 500 ng gave 11,000 revertant colonies compared with about 30 in the controls. In view of

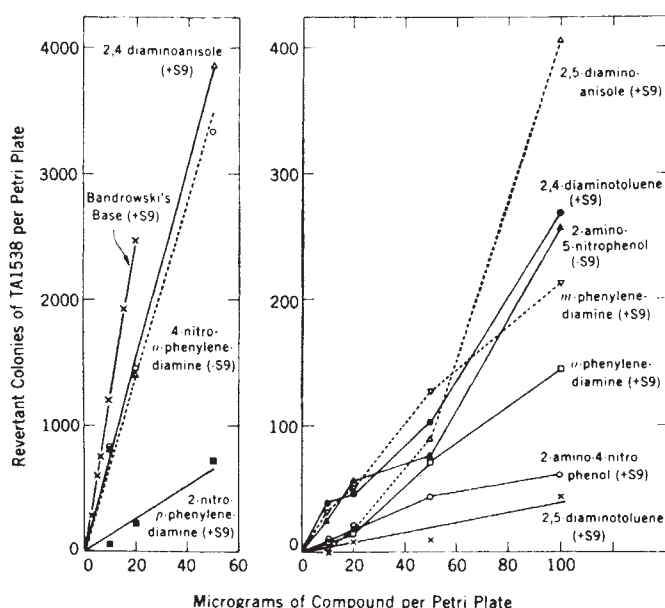


Fig. 3 Dose-response curves of the mutagenicity of chemicals extracted from hair dyes. Revertant colonies were counted after incubating the Petri plates for 48 h at 37 °C with the chemical mutagen and the microsomal activation system (S9) where indicated. (Reproduced, with permission, from Ames *et al.*³⁶.)

the correlation between mutagens and carcinogens any compound which is a potent mutagen is now suspected also of being carcinogenic. Other screening tests in bacteria and mammalian cells have been reviewed by Bridges³⁸.

The first impact of Ames' work should be to eliminate known carcinogens from our food and daily habits so as to reduce the number of carcinogenic events to which we are being exposed—and, incidentally, to let us stop worrying about many other commonly used chemicals which turn out to be harmless. In the long run this research and the work on xeroderma pigmentosum may help us to unravel the exact molecular mechanism by which ultraviolet irradiation and chemicals that revert frame shift and other mutations cause cancer.

What has molecular biology contributed to this work? It has supplied the basic concepts of microbial genetics, mutagenesis, repair and feed-back control; it has supplied the techniques of transferring genetic material from one strain of bacteria to another which Ames used to build up the sensitivity of his system. These concepts and techniques were developed by scientists who set out to interpret fundamental biological processes in physical and chemical terms. Generally the problems were so complex that it took very many years to solve them. At the outset our ignorance was too profound for us to foresee the relationship of our work to human disease, and it became apparent only afterwards. Although we know more now than when we started, molecular biology is still too young a science for it to be clear exactly where it will pay off, whence we may do best if we spread our efforts over a wide field. Since research is the art of the soluble, it is often more profitable to study a basic problem in microorganisms where it can be solved, rather than in mammalian cells which are so much more complex. One of my examples has shown how such an indirect approach has helped to unravel pathogenic events in man. It would be a mistake if all molecular biologists switched to mammalian cells as has recently become the fashion, or as they are being forced to do by policy decisions of supporting agencies. There is a unity of life at the molecular level which implies that anything found to be true in *E. coli* may also hold in man.

In the future, the most important contribution of molecular biology to medical practice may well be genetic engineering, but early hopes that eukaryotic genes could be transcribed and translated in prokaryotes have not yet materialised. Instead, workers may try to see if cultures of animal viruses carrying human genes could be used for the manufacture of therapeutically important human proteins.

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articles

Seeing order in 'amorphous' materials

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Careful analysis of high resolution electron micrographs of amorphous materials, making use of a simple optical simulation technique, provides a means for discriminating between 'meaningful' and 'meaningless' images, and for detecting ordered regions as small as 15 Å in the 'meaningful' ones. Such regions, comprising only about 100 atoms, have been directly observed in 'amorphous' carbon, and it is possible that they exist in a range of other 'amorphous' materials.

The past decade has witnessed a dramatic improvement in the resolving power of high resolution electron microscopes. Atomic planes spaced less than 1 Å apart have been imaged¹, and heavy single atoms have not only been directly observed^{2,3} but their movement between successive exposures followed as well⁴. Routinely attainable point-to-point resolution has come down to around 5 Å and less.

The typical nearest-neighbour distances in most solids are about 2 Å, and many important crystallographic planes have a separation of 3 Å and more. The resolving power of electron microscopes therefore makes it natural to inquire about the possibilities of an atom-by-atom determination of the specimen structure. Such an approach should be particularly fruitful in the case of amorphous (non-crystalline) materials, since in these the conventional structure research based on X-ray, electron or neutron diffraction yields only statistical data averaged over the whole specimen. It describes the probability that an atom A will have a neighbour B at a distance r away, but tells us very little about the local deviations from the average structure.

The first step in the direction of structure determination by direct imaging is a search for small ordered domains within the non-crystalline sample. Such investigations have already yielded much useful information, such as the 'wavy onion' structure of the carbon black particles^{5,6}, and a detailed description of the carbon graphitisation process⁶. In materials other than amorphous carbon the results have been less clear. In amorphous Ge the early dark field (image formed with scattered electrons only) observations of small bright spots about 14 Å in diameter^{7,8}, interpreted in terms of small wurtzite crystallites⁷, have been overtaken by observations of much smaller dark field spots^{9,10} and interpretations denying the presence of any crystallites^{8,10}. Similarly, bright field observations of small patches of fringes⁹⁻¹², interpreted as direct imaging of the crystallite lattice planes^{9,11,12}, have also given rise to interpretations¹⁰ in terms of relatively ordered regions within the continuous random network model^{13,14}, or have simply been discarded as statistical peaking in order within an essentially random image¹⁵.

Meaningful images or just random noise?

The diversity of opinions about the images of amorphous materials is the result of two fundamental difficulties, best illustrated by an analogy. An individual telephone conversation

may remain identifiable even when received over a line with poor frequency response, but once several conversations are superimposed, the total loses all its information content and becomes just noise, in spite of being made up of individually meaningful signals. Similarly, an atom imaged by an electron microscope with a necessarily limited spatial frequency response may remain individually identifiable, but when the images of several such atoms are superimposed, the total may just become a random, unintelligible noise.

Computing the images of various existing structural models for amorphous materials has shown that, for specimens of sufficient thickness, images of completely different models become qualitatively indistinguishable, and image features corresponding to particular structural configurations within the specimen may become completely washed out^{16,17}. This suggests that many experimental images (for which the thickness of the amorphous specimen is usually quite considerable) may indeed be nothing but random, frequency-filtered noise.

Random noise has been the subject of many mathematical studies, mainly in the context of communication engineering.

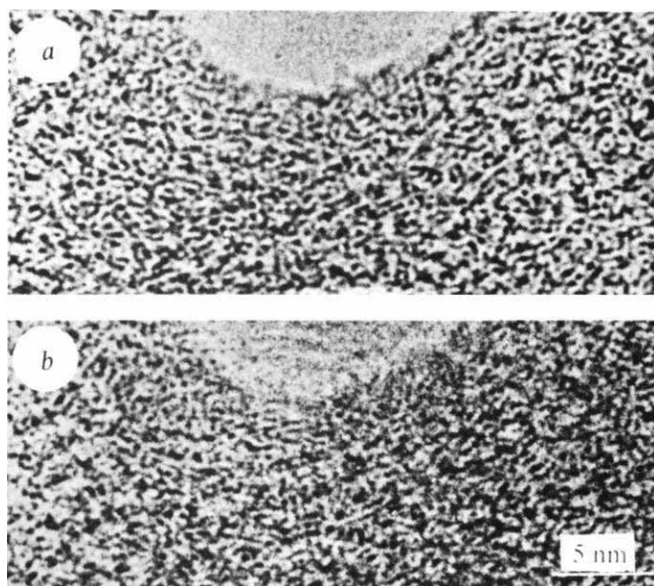


Fig. 1 *a*, Direct reconstruction of an axial bright field micrograph of vapour-deposited amorphous Ge. *b*, Phase-randomised reconstruction of the same image. A Perspex plate, sprayed with transparent lacquer until the droplets (about 0.1 mm in size) just started merging, served as the random phase screen for this and all the other phase-randomised images shown in this article. The resulting surface roughness was enough to ensure that each original patch of fringes was spread over a large area in the reconstruction, rather than merely shifted sideways.

By virtue of the Central Limit Theorem¹⁸, many different mathematical representations of the noise are possible. The one most commonly used is

$$I(\mathbf{r}) = 1 + \sum_n c_n \cos(2\pi \mathbf{q}_n \cdot \mathbf{r} + \phi_n) \quad (1)$$

$I(\mathbf{r})$ is the intensity at a point $\mathbf{r}$ in the image plane, the c_n s are the coefficients defining the image spatial frequency power spectrum, $\mathbf{q}$ is the spatial frequency variable and ϕ_n represents the phase. The summation is taken over all the points of the spatial frequency plane needed to define the image area being considered, and the phases ϕ_n are taken to be distributed randomly in the interval $(0, 2\pi)$. The representation (1) then describes the bright field image of a weak random object (with an unscattered beam of intensity 1). It leads to the prediction of many interesting properties of the random function^{19,20}, some of which are directly applicable to the electron images. For instance, the probability of finding the image intensity I , at an image point selected at random, lying between I and $I+dI$, follows a Gaussian distribution in I . Dark field images are describable by an expression similar to equation (1), although for these the intensity distribution is a negative exponential²¹ rather than a Gaussian one. These distributions can be used to test the randomness of actual electron images¹⁷, but since they treat each image point independently and are therefore incapable of evaluating any patterns within the image, such tests are not particularly sensitive to non-random features. A better test of image randomness that concentrates on groups of image fringes is described here.

An optical simulation technique

It is clear that equation (1), being nothing but a Fourier summation, can be used to represent any image. The random bright field image description only follows upon taking the phases to be random. When the phase information is deliberately removed (by randomising the phases), we obtain an image which has lost all the information on the exact specimen structure the original may have contained. This random image will obviously differ from the original in detail, but it is possible that qualitatively the two images will be identical: every patch of fringes in the original micrograph may be matched by a similar patch of fringes produced in the random image by purely statistical effects. Should this be the case, it is clear that the fringe patches in the original image should not be taken as structurally meaningful. Should the two images display marked differences in the number and regularity of fringe patches present, however, some interesting conclusions about the nature of the specimen should follow from the original.

The randomisation of the phases ϕ_n can be carried out either computationally or in an optical bench¹⁷. The second approach enjoys the advantage of greater simplicity and an ability to handle large image areas. It produces the phase-randomised image in three stages: a diffraction pattern (the spatial frequency power spectrum) of the original micrograph is formed, the phases are randomised by inserting a random phase screen into the diffraction plane, and finally a reverse optical transform leads to the phase-randomised reconstruction. To avoid undesirable interference between the main (unscattered) beam and the inserted phase screen, the main beam is allowed to pass through a hole in the screen. The resulting reconstruction is a simulated 'random' micrograph: although its spatial frequency power spectrum is unchanged from the original, it has become just random noise.

Ordered regions in amorphous Ge, SiO₂ and C

Figures 1, 2 and 3 show how the phase-randomisation technique works in practice. In each figure, image *a* is a direct reconstruction of the original micrograph (recorded with the random

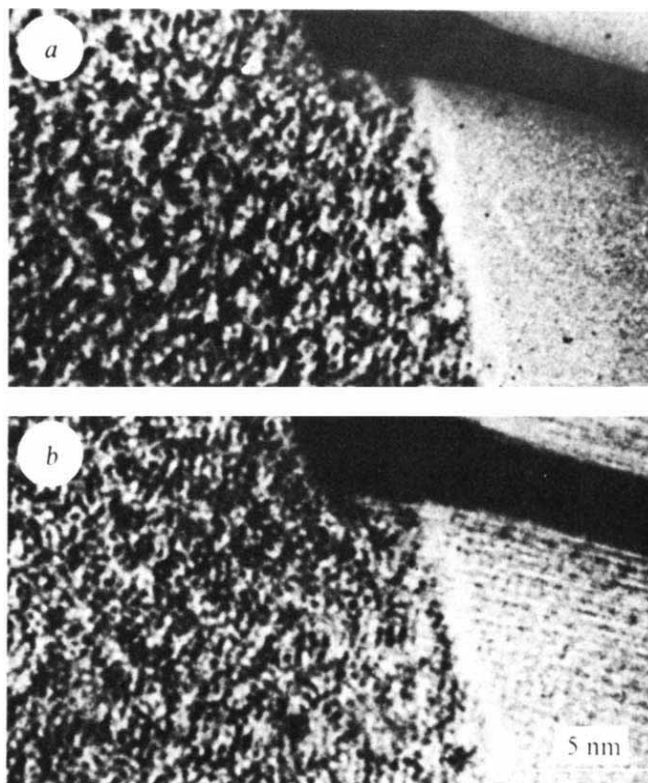


Fig. 2 *a*, Direct reconstruction of an axial bright field micrograph of amorphous SiO₂ prepared by fragmentation of a "Spectrosil" disk. *b*, Phase-randomised reconstruction of the same image.

phase screen removed) and image *b* is its phase-randomised counterpart. The three examples are arranged by the amount of ordered structures visible in the original images. In Fig. 1*a* the 4-Å structures are quite irregular and in Fig. 2*a* some fringes running in the vertical direction appear: even more fringes can be seen in Fig. 3*a*. Such differences may be obvious to the eye, but it is clearly impossible to differentiate between meaningful and fortuitous image features on the basis of such qualitative remarks alone. One can, of course, define objective criteria of fringe regularity such as the signal-to-noise ratio of a group of fringes¹⁷, and then evaluate the significance of the fringes by comparing the experimental values of their signal-to-noise ratio with the theoretical prediction for fringes produced by random effects. Such treatment, however, works with the absolute values of fringe regularity and demands that all the parameters influencing the image formation (such as the amount of inelastic scattering present, astigmatism of the objective lens, specimen drift and so on) be known accurately, which is usually not the case.

By creating an individual reference standard with which the original image can be compared, the phase-randomisation technique altogether avoids the need for such considerations. In the case of amorphous germanium (Fig. 1), for example, the phase-randomised reconstruction is completely different from the original in detail, but it is not difficult to see that qualitatively the two images have remained indistinguishable, with the extent of ordering of the 4-Å structures being about the same in either image. The phase-randomised reconstruction for amorphous carbon (Fig. 3*b*), on the other hand, does not show any ordered image structures comparable to those visible in the original image. Specifically, the patches of relatively straight and parallel fringes spaced about 4 Å apart identifiable at several places in Fig. 3*a* (particularly near the edge of the specimen) find no equivalent in the randomised reconstruction.

Optical diffractograms showing the spatial frequency power spectrum of the direct and phase-randomised images of amorphous carbon are shown in Fig. 4. It can be seen that the phase-randomisation procedure has hardly changed the power

spectrum at all, so that the different appearance of Fig. 3a and b is entirely due to the modification of the phases. It is also worth noting that because the lowest spatial frequencies have passed, together with the main beam, through the hole in the random-phase screen, the coarse image detail has not been altered by the phase randomisation, while the fine image structure has been completely changed by it. As a result of the phase randomisation, the fine structures in all the phase-randomised reconstructions extended well over the specimen edge (see Fig. 3b), and the marker shown in Fig. 2a reappears in Fig. 2b decomposed into its individual spatial frequency components, no longer fitting together as before.

By comparison with the amorphous germanium and amorphous carbon examples, the amorphous silica image (Fig. 2) constitutes an intermediate case. Analysis of the spatial frequencies of the original image by optical diffraction indicates that, due to astigmatism, some directional filtering has taken place. Such filtering will lead to a micrograph with fringes running predominantly in one direction, and directional effects will remain even in the phase-randomised image, showing that the original fringes had no structural meaning whatever. Vertical fringes qualitatively similar to the ones in the direct reconstruction do indeed appear in Fig. 2b, but since they are somewhat more broken up than those visible in Fig. 2a, it is difficult to pronounce judgment on the original image with any certainty.

It should perhaps be emphasised that the intention of this article is to describe a technique for discriminating between meaningful and fortuitous features in high resolution bright field electron micrographs; the three examples used are merely

Fig. 3 *a*, Direct reconstruction of an axial bright field micrograph of vapour-deposited amorphous C. *b*, Phase-randomised reconstruction of the same image.

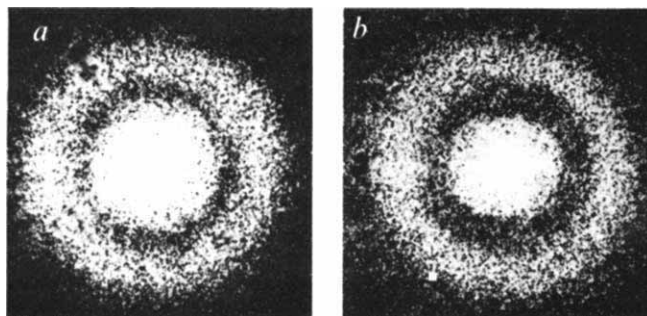
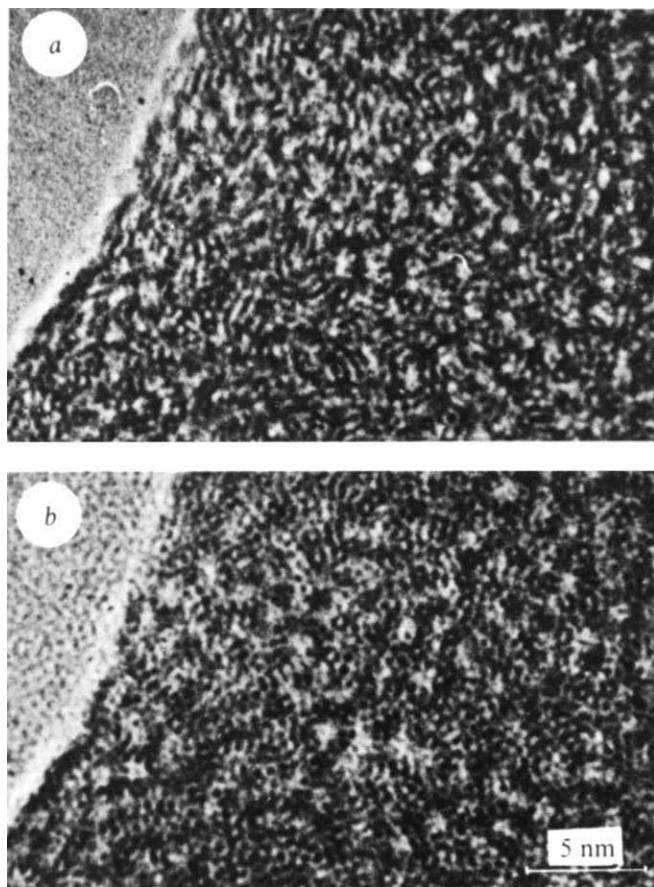


Fig. 4 *a*, Diffraction pattern of Fig. 3a. *b*, Diffraction pattern of Fig. 3b. They show that the micrograph of Fig. 3a was recorded at the broad second transfer interval underfocus ($z_0 \approx -1,400 \text{ \AA}$) with negligible astigmatism ($c_a < 20 \text{ \AA}$). The disappearance of the outer part of the first transfer interval in *b* is due to the opaque edge of the hole in the random-phase screen.

intended to serve as an illustration. It would not be correct to reach any definite conclusion regarding the structure of amorphous materials on the basis of small parts of three electron micrographs, particularly since the structure of these materials is known to depend strongly on the preparation conditions (evaporation rate, substrate temperatures and so on). The possibility of providing a detailed quantitative description of order in such materials in fact constitutes one of the most attractive applications of this technique. Nevertheless, the results obtained with amorphous Ge and C may perhaps be taken as indicative of the results to be expected in these materials. The micrograph of evaporated amorphous Ge (Fig. 1a) contained no image features attributable to well defined ordered regions within the specimen. The fringes seen in the image of evaporated amorphous C were, however, shown definitely not to arise from any fortuitous superposition of the images of distant individual atoms, and must therefore be taken as evidence for small (about 10 to 15 $\text{\AA}$) ordered regions within the material. These regions most probably consist of a few parallel graphite-like planes lying along the direction of the illuminating electron beam.

The absence of any fringes in the Ge micrograph that could be safely attributed to non-random causes does not, however, mean that the specimen contained no ordered regions at all. It can be shown that in a Ge specimen 100 $\text{\AA}$ thick the image of a fully diffracting 15- $\text{\AA}$ crystallite would be obscured by the images of the atoms in the remaining 85 $\text{\AA}$. Only crystallites larger than about 18 $\text{\AA}$ would remain directly visible¹⁷. A similar result also applies for amorphous silica: in a SiO_2 film 100 $\text{\AA}$ thick, only ordered regions larger than about 18 $\text{\AA}$ will remain directly visible, and 15- $\text{\AA}$ ones will be obscured in films thicker than about 50 $\text{\AA}$. In amorphous C this limitation is much less severe, owing mainly to the perfect alignment of the atoms into the graphite planes, which consequently stand out clearly from the amorphous background; 15- $\text{\AA}$ graphite crystallites should remain visible even when embedded in films up to 150 $\text{\AA}$ thick.

The average thickness of the Ge specimen (as determined by monitoring its deposition by a quartz crystal oscillator) was about 100 $\text{\AA}$. It therefore follows that ordered regions smaller than 18 $\text{\AA}$ will not be identifiable. Near the specimen edge (top half of Fig. 1a) the thickness is, however, likely to be smaller, so that the lack of non-random features there places an upper limit of about 15 $\text{\AA}$ on the size of ordered regions possibly present. The amorphous C film was also about 100 $\text{\AA}$ thick, which means that the actual direct observation of the 15- $\text{\AA}$ ordered domains raises no contradiction. In regions much thicker than 100 $\text{\AA}$ the fringes attributable to these domains will be obscured.

To summarise, high resolution electron microscopy has given rise to an approach to the study of the structure of amorphous

materials that is radically different from the conventional methods based on the analysis of diffraction patterns. The first task confronting a researcher using the new approach is the differentiation between structurally meaningful images and those in which too many overlapping atoms obscure the underlying structure. This problem has been solved by the simple technique of optical simulation of random noise with power spectrum identical to the original image. In the image of amorphous Ge examined here the technique shows no evidence of crystallites, and thus places an upper limit of about 15 Å on the size of ordered regions possibly present. In amorphous C, however, small (~ 15 Å) ordered regions have been detected with the technique. It seems quite possible that similar ordering will be discovered in many other types of non-crystalline materials.

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Isotropic cosmic expansion and the Rubin–Ford effect

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We show that the Rubin–Ford (RFR) data, often taken as evidence for large scale anisotropic cosmic expansion, probably only reflect the inhomogeneous distribution of galaxies in the region of the Rubin–Ford sample. The data RFR have presented are consistent with isotropic expansion, an unperturbed galaxy velocity field and hence a low density Universe.

RUBIN, Ford and Rubin¹ have reported on ‘a curious distribution of radial velocities of Sc I galaxies’ which has sometimes been taken as evidence for large scale anisotropic cosmic expansion. The Rubin–Ford sample consists of 74 galaxies with measured radial velocities (corrected for galactic rotation) and apparent magnitudes (as determined by Zwicky) in the ‘window’ $14.0 \leq m_{Zw} \leq 15.0$. These galaxies were chosen from a larger sample of 200 galaxies, selected from Palomar Sky Survey plates and classified as being of type Sc I according to the David Dunlap Observatory system².

RFR note in ref. 1 that the apparent velocity distribution of their sample is distinctly anisotropic. In one region of the sky (called region I), most of the sample galaxies have radial velocities $v_I \simeq 4,950$ km s^{−1}, while in the opposite region (called region II) most of the sample galaxies have radial velocities $v_{II} \simeq 6,400$ km s^{−1}. The mean apparent magnitudes of sample galaxies in the two regions differ by only 0.1 mag and the mean apparent diameters differ by only 14%. Since Sc I galaxies are thought to be good ‘standard candles’, RFR make the suggestion that their data may be evidence for anisotropic cosmic expansion. If true, this would have important cosmological implications, especially in view of the apparent isotropy of the microwave background radiation. Our purpose in this article is to point out that the Rubin–Ford data are consistent with isotropic expansion and indeed, the effect might have been expected on the hypothesis that the expansion is isotropic.

Inhomogeneities

The idea underlying our argument is illustrated in Fig. 1 where we have plotted the positions of Sc I galaxies in the Rubin–Ford

sample with declinations in the range $20^\circ < \delta < 42^\circ$. We have also plotted all galaxy clusters in this declination band with radial velocities $< 10,000$ km s^{−1} and tabulated in Noonan’s list of clusters with published redshifts³. Those clusters classified by Abell⁴ as being ‘rich’ are represented by a circle of radius $10 h^{-1}$ Mpc (where h is the Hubble constant measured in units of 100 km s^{−1} Mpc^{−1}). From Peebles’ Abell cluster–galaxy correlation function⁵, this radius corresponds to a rich cluster density contour at some 3 times the background density. The other clusters were plotted with radii of $7 h^{-1}$ Mpc unless they seemed, from an inspection of Zwicky’s catalogue⁶ to be particularly small or poor. In that case they were plotted with radii of $4 h^{-1}$ Mpc. The sizes are only meant to give a rough indication of the distribution of galaxies in the vicinity of clusters. To estimate the completeness of Noonan’s list in this region, we have checked the Zwicky catalogue and find only one (or possibly two) additional clusters which are comparable with those of known radial velocities.

The correlation between the positions of Rubin–Ford Sc I galaxies and clusters in Fig. 1 is quite striking. Only one of the Sc I galaxies is significantly isolated from any cluster. RFR point out that some 60% of their sample galaxies lie outside the cores of known clusters. Nonetheless, it is apparent from Fig. 1 that they are associated with the Perseus–Pisces complex and Abell’s⁷ supercluster no. 6 (including Coma and A1367). This would be expected on the ‘cloud picture’ of the galaxy distribution^{8–10}. Figure 1 suggests to us that the apparent anisotropy in the radial velocities of Sc I galaxies may reflect only the inhomogeneities in the distribution of galaxies in the region of the Rubin–Ford sample.

Of course, we have picked the declination band where the effect is most dramatic to illustrate the basic idea underlying our hypothesis. To show that the correlation of Sc I galaxies with clusters is a general phenomenon, we present in Table 1 the number of Rubin–Ford sample galaxies and Noonan clusters in the high and low velocity bands of regions I and II for the whole sky north of $\delta = -3^\circ$. We note that the ratio of the number of clusters to Sc I galaxies in the low velocity band of region I is nearly the same as this ratio for the high velocity band of region II, as expected on our hypothesis. We also note that there is an apparent deficit of high velocity Sc I galaxies in

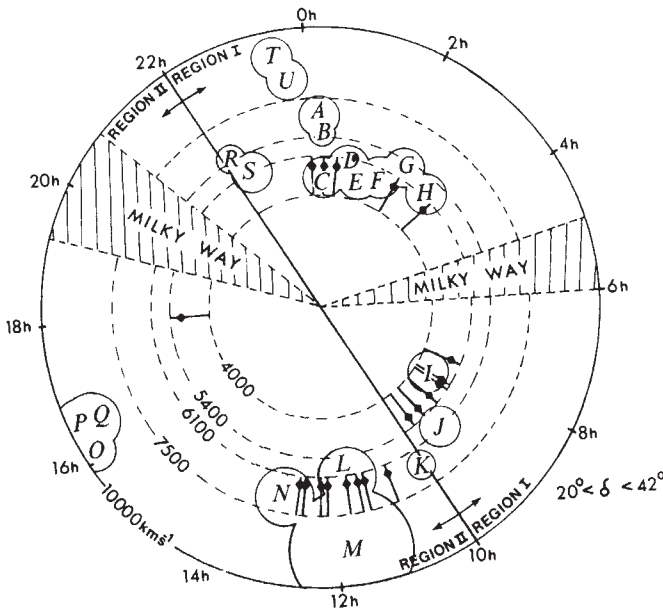


Fig. 1 Clusters (○) and Rubin-Ford Sc I galaxies (—♦—) with $20^\circ < \delta < 42^\circ$. Clusters: A, B, Anonymous; C, Perseus C₂; D, Z501-5; E, Z501-5; F, Z522-9; G, A347; H, A426, Z540-1 (Perseus); I, Z119-1 (Cancer); J, A779, Z181-17; K, Z211-58; L, A1367, Z127-2; M, Abell Supercluster no. 6; N, A1656, Z160-1 (Coma); O, A2162, Z167-8; P, A2199, Z224-12; Q, A2197, Z224-12; R, Z513-2; S, Z514-3; T, A2634, Z476-5; U, A2666, Z476-5. Numbers preceded by A are entries in Abell's catalogue⁴ and numbers preceded by Z are entries in Zwicky's catalogue⁶. All radial velocities are from Noonan⁹ except C which is from Zwicky⁹ and M which is from Abell⁷. The circular scale gives RA (h), the radial scale gives velocity (km s⁻¹).

region I. As the high velocity clusters in region I are among the poorest in the sample, however, this fact should not be given equal weight.

The two-cloud model

Figure 1, together with the Rubin-Ford comment that most of their sample galaxies in region I have velocities near 4,950 km s⁻¹ and those in region II have velocities near 6,400 km s⁻¹, suggests that we idealise to a model in which galaxies lie in two 'clouds' with those velocities. We shall refer to this model as the 'two-cloud model' and denote the low velocity cloud I and the high velocity cloud II. Of course, the applicability of the model results from the particular magnitude window chosen by RFR and the distribution of clusters within it (compare Fig. 1). In what follows, we shall assume (1) that the Sc I galaxies in both clouds are drawn from a universal urn, that is from the same luminosity function and size distribution, and (2) that there is no anisotropy in the cosmic expansion, that is, distances are given by Hubble's law with a direction independent Hubble constant. We shall then endeavour to show that this model is consistent with the data presented in ref. 1 by RFR; we shall 'predict' (1) the 0.1 apparent magnitude difference between low and high velocity sample galaxies and (2) the 14% difference in mean angular diameters.

First, we compute the mean apparent magnitudes of high and low velocity galaxies expected on the two-cloud model in a manner suggested by Sandage and Tammann's discussion of the Rubin-Ford effect¹¹. We adopt the luminosity function for Sc I galaxies suggested by van den Bergh² and Sandage and Tammann¹¹; a Gaussian with mean absolute (photographic) magnitude

$$\bar{M} = -20.0 + 5 \log h \quad (1)$$

and dispersion

$$\sigma(M) = 0.50 \text{ mag} \quad (2)$$

With this luminosity function we compute mean apparent magnitudes

$$\bar{m}_I = 14.17, \bar{m}_{II} = 14.30 \text{ (model)} \quad (3)$$

for the low and high velocity clouds respectively. The calculation is illustrated in Fig. 2 and is of course independent of h and the numbers of galaxies in the clouds. We have adopted a photometric correction of -0.1 mag to Zwicky magnitudes as suggested by Sandage and Tammann¹¹. The predicted mean apparent magnitudes (3) are to be compared with the mean apparent magnitudes of the Rubin-Ford sample, corrected for galactic obscuration

$$\bar{m}_I^c = 14.20 \pm 0.07, \bar{m}_{II}^c = 14.30 \pm 0.06 \quad \text{(Rubin-Ford data)} \quad (4)$$

The agreement is remarkably good. We note that the predicted magnitudes are somewhat sensitive to the adopted magnitude window, but that their near equality is not. This near equality is mainly a consequence of the fact that the one-magnitude window chosen by RFR is comparable with the dispersion in the absolute magnitudes of Sc I galaxies.

Next we estimate the ratio of the mean angular diameters of the low and high velocity galaxies expected on the two-cloud model. We shall assume that galaxy diameters d and luminosities L are related by

$$d \propto L^{(1-\epsilon)/2} \quad (5)$$

where ϵ is a parameter to be discussed presently. From (5) and the relationship between distances, luminosities and apparent and absolute magnitudes, it follows that the ratio of the mean angular diameters for galaxies in clouds I and II is

$$\bar{\theta}_I/\bar{\theta}_{II} = (v_{II}/v_I)^\epsilon [1 + \frac{1}{5}(1-\epsilon)(\bar{m}_{II} - \bar{m}_I) \ln(10) + \dots] \quad (6)$$

to first order in $(\bar{m}_{II} - \bar{m}_I)$. For the two-cloud model appropriate to the Rubin-Ford data this becomes

$$\bar{\theta}_I/\bar{\theta}_{II} \simeq (1.29)^\epsilon (1.06 - 0.06\epsilon) \text{ (model)} \quad (7)$$

with the mean apparent magnitude difference calculated above (equation (3)). The case $\epsilon = 0$ corresponds to luminosities uncorrelated with mean surface brightness as Hubble¹² found to be appropriate for elliptical galaxies, and the extreme case $\epsilon = 1$ corresponds to luminosities uncorrelated with diameters.

Unfortunately, it is rather difficult to determine the luminosity-diameter relation for Sc I galaxies because one requires a large sample of galaxies, all at the same distance. It would be surprising if the value of ϵ appropriate to Sc I galaxies did not lie in the range (0,1) bracketed by the extreme cases mentioned above. We expect it to lie nearer the value $\epsilon = 0.23$ found by Heidman *et al.*¹³ to fit the data on spiral galaxies in the Virgo cluster best. This is similar to the value $\epsilon = 0.17$ found by Holmberg¹⁴ to fit the data on galaxies of all morphological types. In any case, the dependence of equation (7) on ϵ is rather weak in this neighbourhood. With $\epsilon = 0.23$, equation (7) gives the ratio

$$\bar{\theta}_I/\bar{\theta}_{II} \simeq 1.11 \text{ (model)} \quad (8)$$

Table 1 Numbers of Rubin-Ford Sc I galaxies and Noonan clusters north of $\delta = -3^\circ$

Region	Low velocity (4,000–5,400 km s ⁻¹)		High velocity (6,100–7,500 km s ⁻¹)	
	Galaxies	Clusters	Galaxies	Clusters
I	20	5	2	5
II	1	0	12	3

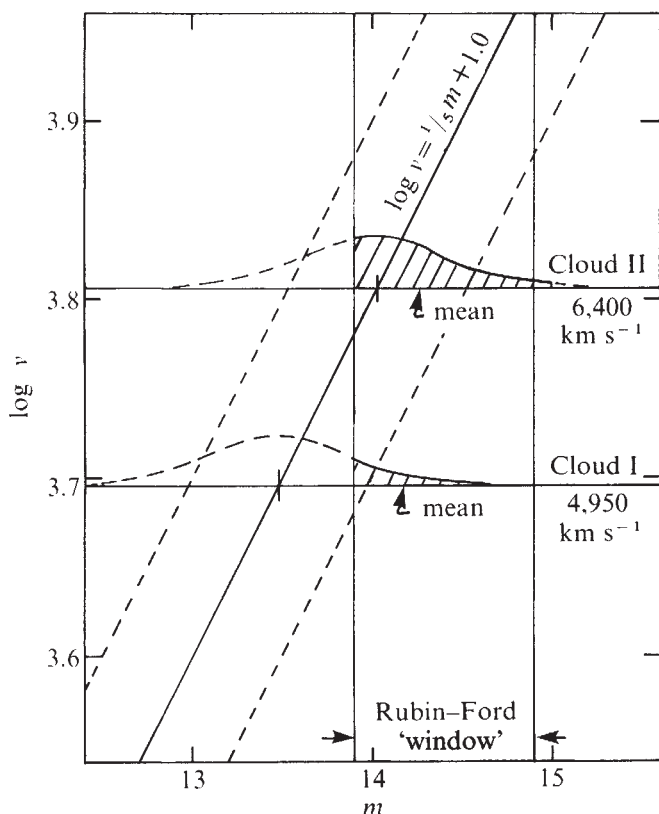


Fig. 2 Graph illustrating the calculation of mean apparent magnitudes for low and high velocity galaxies on the two-cloud model. The dashed diagonal lines are $\sigma = 0.50$ mag away from the mean velocity-magnitude line. All velocities are in km s^{-1} .

which is to be compared with the ratio

$$\bar{\theta}_I/\bar{\theta}_{II} = 1.14 \pm 0.10 \quad (\text{Rubin-Ford data}) \quad (9)$$

quoted by RFR for their sample. In view of the large scatter in the angular diameters of Rubin-Ford galaxies, the agreement between equations (8) and (9) is entirely satisfactory.

The local velocity field

RFR have also pointed out that there is an apparent anisotropy in the radial velocities of the bright Sc I and Sc I-II galaxies studied by van den Bergh² and that the effect is similar in magnitude and in direction to that in the Rubin-Ford sample. That is, the van den Bergh galaxies in the Rubin-Ford region I seem to yield a Hubble constant similar to that of Rubin-Ford galaxies in region I but significantly smaller than that of both van den Bergh and Rubin-Ford galaxies in region II. RFR argue from this that inhomogeneity scales of at least $70 h^{-1}$ Mpc are required by the hypothesis of isotropic expansion. How can this be reconciled with our assumption of isotropic expansion and inhomogeneities on the more reasonable scale of $15 h^{-1}$ Mpc? We have re-examined the data on van den Bergh's sample of Sc I and Sc I-II galaxies and find that the anomaly arises mainly from those galaxies lying within $\sim 20^\circ$ of the centre of the Ursa Major complex. This is a small part of Rubin-Ford region I which we shall refer to as subregion U. Most of the van den Bergh galaxies in Rubin-Ford region II lie within $\sim 20^\circ$ of the Virgo cluster, a subregion which we shall denote by V.

Because of the unknown distribution of galaxies behind the Ursa Major and Virgo complexes, a detailed calculation is out of the question. The following remarks, however, strongly suggest that the anomaly may indeed be caused by inhomogeneities on the smaller scales: (1) the mean velocities of van den Bergh Sc I and Sc I-II galaxies lying in subregion U is very

close to the mean velocity of both de Vaucouleurs¹⁵ groups and Turner-Gott¹⁶ groups lying in that subregion; (2) the mean velocity of van den Bergh Sc I and Sc I-II galaxies lying in subregion V is $\sim 2,000 \text{ km s}^{-1}$, almost $1,000 \text{ km s}^{-1}$ larger than the mean recessional velocity of the Virgo cluster. The apparent discrepancy could therefore be reconciled with isotropic expansion and moderate inhomogeneities on a theme similar to the two-cloud model if the Virgo complex in fact extends for $15 h^{-1}$ Mpc or so in the radial direction. Another possibility is simply that the velocities of some of the Sc I and Sc I-II galaxies in subregion V are dominated by the core of the Virgo cluster and are therefore not indicators of their distance. Indeed, according to Tammann¹⁷ the van den Bergh galaxies having NGC numbers 4254, 4303, 4321 and 4535 lie in the core of the Virgo cluster. If these galaxies are put at the distance of the Virgo cluster, the anomaly between subregions U and V nearly disappears, and the remaining difference might be accounted for along the lines mentioned above.

Discussion

The agreement between the 'predictions' of the two-cloud model and the data reported by RFR in ref. 1 is encouraging. We emphasise again that the applicability of the model results from the particular, narrow magnitude window chosen by RFR and the distribution of galaxies (as suggested by the distribution of clusters) within that window. Unfortunately, the radial galaxy distribution at these distances is not yet accurately known over large parts of the sky and that is, of course, the reason for our choosing clusters of galaxies as a reflection of the distribution of galaxies. Recently, Chincarini and Rood have studied the radial velocity distribution of individual galaxies in the direction of the Coma cluster and its surroundings¹⁸ and the Perseus-Pisces complex (G. Chincarini, personal communication) and find distributions quite consistent the cloud picture depicted in Fig. 1. We note that the density enhancements required to produce the Rubin-Ford effect on the hypothesis of isotropic expansion are of ~ 1 on radial scales $\sim 15 h^{-1}$ Mpc (corresponding to the Rubin-Ford magnitude window). Studies of the distribution of galaxies within the Local Supercluster¹⁹ indicate that the required matter gradients are not atypical. On the hypotheses of uniform expansion and a uniform matter distribution on the very largest scales, the Rubin-Ford effect should of course vanish. Support for this comes from Evans and Hart²⁰ who report no anisotropy in the angular diameter-redshift relationship for elliptical galaxies with radial velocities in the range $1,200 \text{ km s}^{-1}$ to $25,000 \text{ km s}^{-1}$.

The Rubin-Ford effect should appear, on our hypothesis, in the distribution of any 'standard candles' in a one-magnitude window scaled to the RFR window (that is scaled by the difference in the mean absolute magnitudes of Sc I galaxies and that of the other standard candle). Le Denmat and Vigier²¹ have suggested that type I supernovae may be appropriate standard candles and have discovered an effect similar to the RFR effect in their distribution. Considering, however, the methods used in searching for supernovae, their apparent distribution is almost guaranteed to coincide with the distribution of galaxy clusters, and would have been expected to exhibit an anisotropic velocity distribution on our hypothesis. We have re-examined the data on type I and possible type I supernovae^{22, 23} in the magnitude windows $15.3 \leq m \leq 16.3$ and $15.7 \leq m \leq 16.7$. These correspond respectively to supernovae mean absolute magnitudes 1.4 mag (ref. 24) and 1.8 mag (ref. 25) fainter than Sc I galaxies (see equation (1)). We do indeed find that most of these supernovae have occurred in galaxies which are members of the Coma, Cancer and Perseus-Pisces clusters (see Fig. 1). Jaakola *et al.*²⁶ have also noted an effect similar to the Rubin-Ford effect in the distribution of compact galaxies with absorption spectra. It is not clear, however, that these objects represent standard candles in the sense required to evaluate our hypothesis.

In summary, we have shown that the Rubin-Ford data are consistent with the hypothesis of isotropic cosmic expansion. In

view of the similarity of Sandage and Tammann's¹¹ velocity-magnitude relationship (see their Fig. 4) in the supergalactic centre and anticentre directions, a peculiar anisotropy in the absolute magnitudes of Sc I galaxies which just compensates any anisotropy in the velocities is required if the cosmic expansion is not to be isotropic. We consider such a conspiracy to be less plausible than the hypothesis of isotropic expansion. The issue cannot be finally decided, however, until radial velocities and radial distribution of other kinds of matter at these distances are more accurately known. This will require velocity and magnitude data for several kinds of standard candles ('standard candle' meaning, in this context, a class of objects with an absolute magnitude dispersion $\lesssim 0.50$ mag, corresponding to the inhomogeneity scale $15 h^{-1}$ Mpc at these distances).

Of course, we do not expect the cosmic expansion to be perfectly isotropic in the direction of clusters and clouds of galaxies because these matter inhomogeneities will have perturbed the expansion to some extent. Since the perturbed galaxy velocities near an inhomogeneity are determined by the absolute amount of matter in the inhomogeneity, its mass and hence the mean matter density of the Universe can, in principle, be determined once the galaxy distribution and velocity field near the inhomogeneity are mapped out^{19, 27-29}. As we have shown, the Rubin-Ford data are consistent with an unperturbed galaxy velocity field and hence a low density Universe. Paradoxically, the apparent anisotropy of the radial velocities reported by RFR in ref. 1 may impose a rather strong upper limit to the mean matter density of the Universe. To see this, refer back to Fig. 1. Suppose now that matter is decelerated towards the centre of the supercluster at RA 12 h. Then the matter on this side of the supercluster centre will be closer to us than the distance assigned on the two-cloud model from Hubble's law. The galaxies in this cloud would then appear too bright to be consistent with the Rubin-Ford data. At this stage, we refrain

from carrying out a calculation of the mean matter density on this basis because of the uncertainties inherent in the limited data at hand. In view of the importance of this parameter and the hypothesis of isotropic expansion to cosmology, it would be very desirable to have more velocity and magnitude data for galaxies at these distances.

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Fossil hominids from the Laetolil Beds

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Remains of 13 early hominids have been found in the Laetolil Beds in northern Tanzania, 30 miles south of Olduvai Gorge. Potassium-argon dating of the fossiliferous deposits gives an upper limit averaging 3.59 Myr and a lower limit of 3.77 Myr. An extensive mammalian fauna is associated. The fossils occur in the upper 30 m of ash-fall and aeolian tuffs whose total measured thickness is 130 m.

THE fossil-bearing deposits referred to variously as the Laetolil Beds, Garusi or Vogel River Series lie in the southern Serengeti Plains, in northern Tanzania, 20-30 miles from the camp site at Olduvai Gorge.

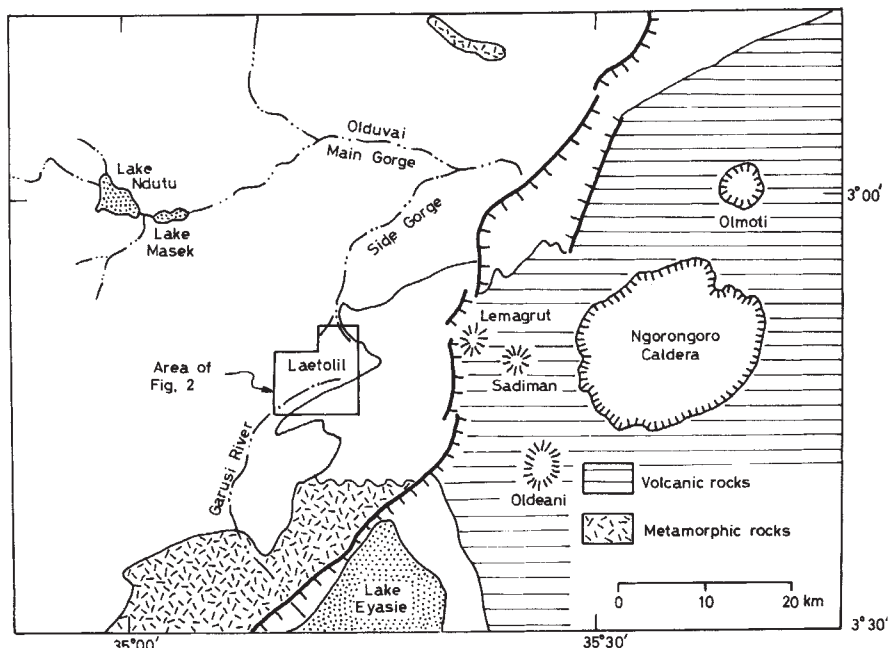
Fossils have been collected from the area on several occasions, the largest collection being made by L. Kohl-Larsen in 1938-39, who also found a small fragment of hominid maxilla which was named *Meganthropus africanus* by Weinert¹. L. S. B. Leakey and M.D.L. visited the area in 1935 and in 1959, while a day trip was made in 1964 in company with R. L. H. The faunal material recovered on these occasions was all collected before the advent of isotopic dating and the age of the fossils remained uncertain until potassium-argon dating was carried

out on samples of biotite obtained during the 1975 field season. In 1974, however, lava flows which unconformably overlie the Laetolil Beds had been dated by G. H. C. at 2.4 Myr.

We now report evidence that fossiliferous deposits of several different ages exist in the Laetolil area and that specimens found on the surface are not necessarily derived from the same beds. There are, however, noticeable variations in the colour and physical condition of the surface fossils which provide indications of their origin.

Discrepancies in the fauna were noted by Dietrich² and Maglio³ who both postulated faunal assemblages of two different ages. In view of this, it was proposed for a time that the name Laetolil should be abandoned in favour of the more generalised term Vogel River Series, based on the colloquial German name for the Garusi river, which abounds in bird life. As the early fossiliferous deposits here referred to as the Laetolil Beds are not confined to the Garusi valley, this change of name seems unnecessary. Furthermore, the name Laetolil embraces a larger area, because it is the anglicised version of the Masai name (laetoli) for *Haemanthus*, a red lily that is abundant in the locality. The Laetolil Beds, *sensu stricto*, form a discrete unit, distinguishable from later deposits, and M.D.L. considers that the original name proposed by Kent

Fig. 1 Map of the southern Serengeti and volcanic highlands.



in 1941⁴ should be retained for this part of the sequence.

The relationship of the Laetoli to the Olduvai Beds had been under discussion for some years, but in 1969 R. L. H. noted that they underlay bed I at the Kelogi inselberg in the Side Gorge and established that they antedated the Olduvai sequence. This has been further confirmed when tuffs correlatable with bed I as well as an earlier, fossil-bearing series of tuffs were found to lie unconformably on the Laetoli Beds.

Interest in the area was renewed in 1974 after the discovery by George Dove of fossil equid and bovid teeth in the bed of the Gagjinger river, which drains into Lake Masek at the head of Olduvai Gorge. These fossils were found to be eroding from relatively recent deposits, probably the beds named Ngaloba by Kent⁴. Exposures of the Laetoli Beds, not hitherto seen by M.D.L., were found to the east of the Gagjinger river, at the headwaters of the Garusi river and of the Olduvai Side Gorge (referred to as Maramba by Kohl-Larsen⁵). Several fossils, including a hominid premolar, were found at these localities and subsequent visits yielded further hominid remains.

The possibility of establishing the age of the hominid fossils from the Laetoli Beds by radiometric dating and of clarifying the discrepancies in the faunal material led to a 2-month field season during July and August 1975. Samples from the fossiliferous horizons, collected by R. L. H., have now been dated. On the basis of these results the hominid remains and associated fauna can be bracketed in time between 3.59 and 3.77 Myr.

No trace of stone tools or even of utilised bone or stone was observed in the material from the Laetoli Beds, although handaxes and other artefacts occur in conglomerates which are present in certain areas and which are unconformable to the Laetoli Beds.

Stratigraphy of Laetoli area

The bulk of the faunal remains and all of the hominid remains were found within an area of about 30 km² at the northern margin of the Eyasi plateau and in the divide between the Olduvai and Eyasi drainage systems (Fig. 1).

Kent studied this area as a member of L. S. B. Leakey's 1934–35 expedition, and his short paper is the only published description of the stratigraphy. He recognised three main subdivisions of the stratigraphic sequence, which overlies the metamorphic complex of Precambrian age. The lower unit he named the Laetoli Beds and the upper the Ngaloba Beds. The middle unit consists of olivine-rich lava flows and agglomerate, which are much closer in age to the Laetoli Beds than to

the Ngaloba Beds. Kent briefly noted the local occurrence of tuffs younger than the lavas and older than the Ngaloba Beds. He described the Laetoli Beds as subaerially deposited tuffs, and he gave 30 m as an aggregate thickness in the vicinity of Laetoli. The Ngaloba Beds he described as tuffaceous clays, and he gave a thickness of about 5 m at the type locality. Pickering mapped this area as part of a 1:125,000 quarter degree sheet⁶ and extended the known occurrence of the Laetoli Beds. He also recognised that at least some of the tuffs are of nephelinite composition.

This picture was modified considerably by stratigraphic work in 1974 and 1975. The Laetoli Beds proved to be far thicker and more extensive than previously recognised. The thickest section, 130 m, was measured in a valley in the northern part of the area (geological localities A to C, Fig. 2). The base is not exposed and the full thickness of the Laetoli Beds here is unknown. Sections 100–120 m thick and representing only part of the Laetoli Beds were measured about 10 km south-east of Laetoli. The Laetoli Beds are 15–20 m thick at a distance of 25–30 km to the south-west and 10–15 m thick at Lakes Masek and Ndutu, 30 km to the north-west. The Laetoli Beds are tuffaceous sediments, dominantly of nephelinite composition.

The lavas and agglomerates noted by Kent overlie an irregular surface deeply eroded into the Laetoli Beds. The lavas were erupted from numerous small vents to the south, south-west and west of Lemagrut volcano. Although designated as nephelinite by Kent, the flows proved to be vogesite, a highly mafic lava with interstitial alkali feldspar and phlogopitic biotite. The lavas have reversed polarity as determined by field measurements (personal communication from A. Cox). A sample of lava from geological locality D (Fig. 2) gave K–Ar dates of 2.38 ± 0.5 Myr and 2.43 ± 0.7 Myr (Table 1).

The 130-m section of the Laetoli Beds (Fig. 3) is divisible into an upper half consisting largely of wind-worked, or aeolian, tuff⁷ and a lower half consisting of interbedded ash-fall and aeolian tuff with minor conglomerate and breccia. Tephra in the lower half are nephelinite, whereas nephelinite and melilitite are subequal in the upper half. Between these two divisions is a distinctive biotite-bearing coarse lithic-crystal tuff 60 cm thick. Hominid remains and nearly all of the other vertebrate remains are confined to the uppermost 30 m of aeolian tuffs beneath a widespread pale yellow vitric tuff 8 m thick (tuff d of Fig. 3). Several other marker tuffs, between 1 m and 30 cm thick, can be used for correlating within the fossiliferous 30-m thickness of sediments. The three prominent marker tuffs, designated a, b, and c (Fig. 3, column 2), can be recognised

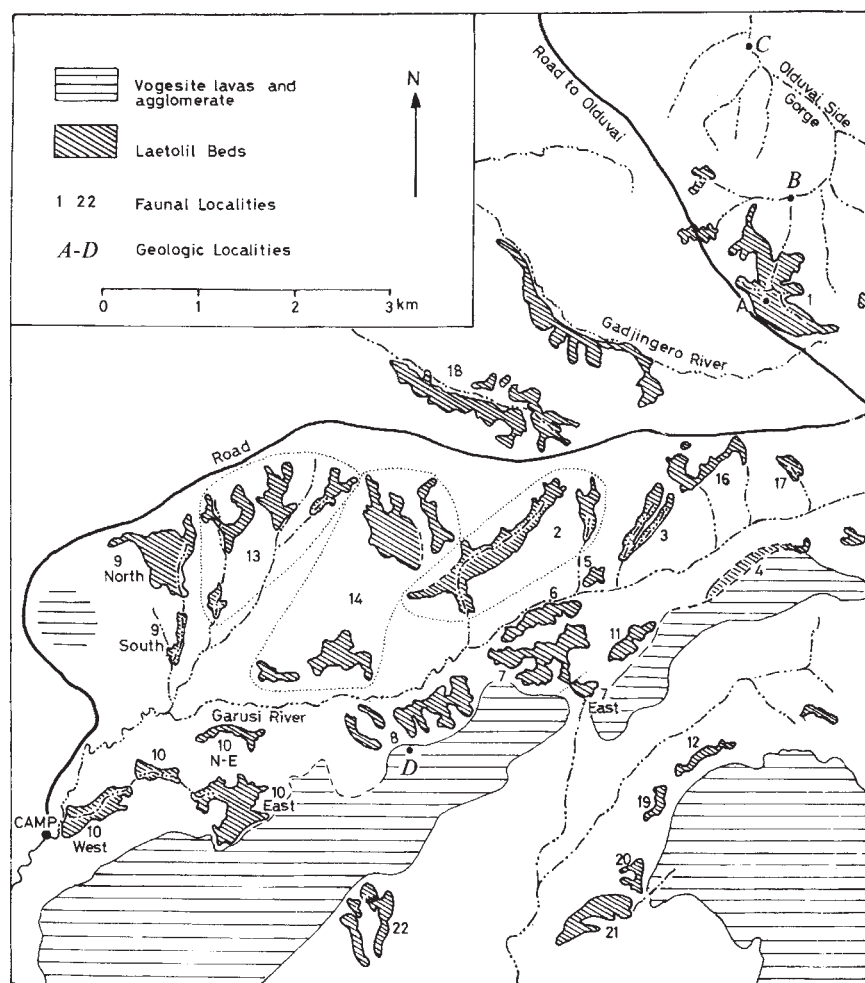


Fig. 2 Map of the Laetolil area showing the fossil beds.

throughout the area shown in Fig. 2. Widespread horizons of ijolite and lava (mostly nephelinite) xenoliths are at several levels in the fossiliferous part of the section and assist in correlating. Biotite is common in some of the ijolite.

Additional fossiliferous deposits, of mineralogical affinity to the Laetolil Beds and 10–15 m thick, lie between tuff *d* and the bed I (?) tuffs at several places. They are locally separated from tuff *d* and the underlying aeolian tuffs of the Laetolil Beds by an erosional surface with a relief of 8 m. These sediments comprise water-worked tuffs, aeolian tuffs, clay-pellet aggregate of aeolian origin and limestone. The tephra are of phonolite and nephelinite composition. It is not yet clear whether or not these sediments should be regarded as part of the Laetolil Beds.

Beds I and II are represented by sedimentary deposits that lie stratigraphically between the lava and the Ngaloba Beds. The bed II deposits locally contain fossils and artefacts.

Sadiman volcano, about 15 km east of the fossiliferous exposures, seems to have been the eruptive source of the Laetolil Beds. The one K–Ar date, of 3.73 Myr, obtained from Sadiman lava, fits with the K–Ar dates on the Laetolil Beds presented here. This date was published previously as K–Ar 2238 (ref. 8), where it was incorrectly assigned to Ngorongoro because of a mistake in listing the sample numbers.

Potassium–argon dating

The vogesite lava is composed of approximately 85–90% olivine and augite. The remainder is principally anorthoclase in the groundmass together with a very small amount of phlogopitic biotite. These two minerals proved too fine-grained and sparse for effective separation, and whole-rock samples were used for dating.

Table 1 K–Ar dates from the Laetolil area

Sample	KA no.	Dated material	Sample weight (g)	% K	mol g ⁻¹ ⁴⁰ Ar radiation × 10 ⁻¹¹	% ⁴⁰ Ar atmosphere	Age yr (Myr)	Remarks
Vogesite lava, location D	2835	Whole rock	10.06588	1.013	0.419	74.0	2.38 ± .05	Unconformably overlying Laetolil Beds
	2837R	Whole rock	11.27408	1.013	0.427	84.4	2.43 ± .08	
	2929	Whole rock	1.98629	6.96 ± .03	4.118	77.5	3.41 ± .08	Treated with dilute HCl
Tuff c, location A	2977	Whole rock	2.544	6.98 ± .04	4.462	78.5	3.68 ± .09	Treated with warm dilute acetic acid
	2979	Whole rock	1.94298	6.95 ± .04	4.454	79.1	3.69 ± .10	
Xenolithic horizon, location A	2930	Whole rock	3.07730	7.58 ± .02	4.771	50.8	3.62 ± .09	Treated with dilute HCl
	2930R	Whole rock	1.83909	7.58 ± .02	4.733	39.7	3.59 ± .05	
Ash-fall tuff, location B	2932	Whole rock	1.05978	6.00 ± .1	3.996	75.0	3.82 ± .16	Crushed, hand picked, treated with dilute HCl
	2938	Whole rock	1.42092	6.49 ± .04	4.182	78.8	3.71 ± .12	

⁴⁰K/K = 1.18 × 10⁻⁴; ⁴⁰K_λ = 5.480 × 10⁻¹⁰ yr⁻¹; ⁴⁰K_{λβ} = 4.905 × 4.905 × 10⁻¹⁰ yr⁻¹; ⁴⁰K_{λε} = 0.575 × 10⁻¹⁰ yr⁻¹.

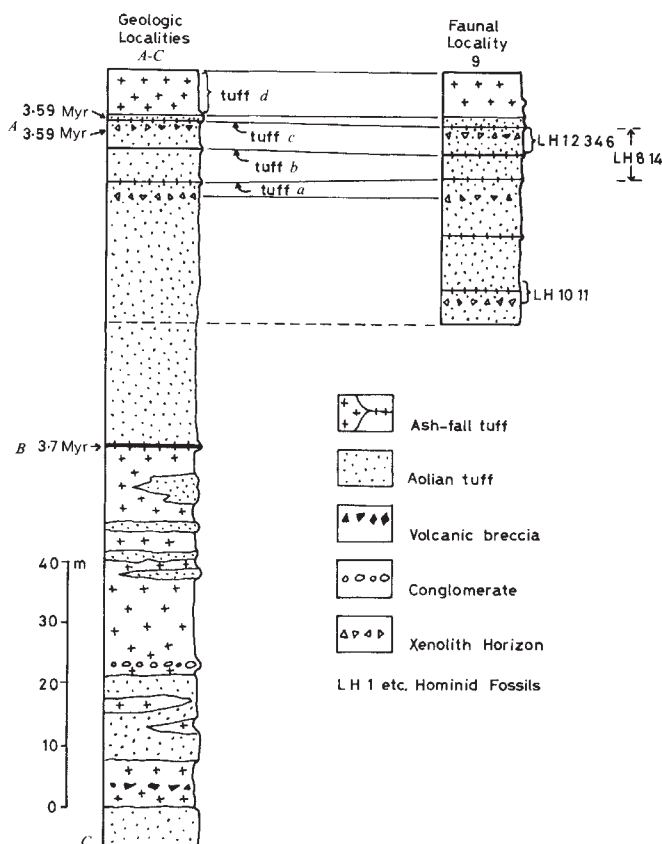


Fig. 3 Stratigraphic column of the Laetolil Beds showing the positions of the dated tuffs and hominid fossils.

In addition to the vogesite lava unconformably overlying the Laetolil Beds, three of the tuffaceous layers within the Laetolil Beds have been dated by the conventional, total degassing K-Ar method (Table 1), and one of these tuffaceous layers has also been dated by the $^{40}\text{Ar}/^{39}\text{Ar}$ method, using incremental heating.

Tuff *c* is the uppermost of the dated horizons, lying near the top of the fossiliferous deposits. It is a widespread crystal-lithic air-fall tuff cemented with calcite and generally 10–15 cm thick. Abundant biotite crystals 1–2 cm in diameter occur in the upper part of the tuff, which is composed of nepheline and milelitite. The dated crystals were hand-picked from two outcrops of the tuff at locality *A* (Figs 2 and 3). The cementing calcite adhering to and interleaving the biotite books was removed by treatment with dilute HCl for a few minutes on one sample and with warm dilute acetic acid on two other samples. This treatment was found to have negligible deleterious effects on biotite standard samples. The three dates obtained for tuff *c* (3.41 ± 0.08 Myr, 3.68 ± 0.09 Myr, and 3.69 ± 0.10 Myr) have about equal precision so were averaged to give a date of 3.59 Myr.

Two conventional K-Ar dates and one $^{40}\text{Ar}/^{39}\text{Ar}$ were obtained from a single biotite crystal from a xenolithic horizon at locality *A* (Figs 2 and 3) approximately 1–2 m below the youngest tuff dated (tuff *c*). This horizon lies within the upper part of the fossiliferous beds and is distinguished by its ejecta of ijolite xenoliths together with nepheline lava xenoliths. Although the biotite occurs in some of the ijolite clasts, a large single free crystal picked from the tuff was used for dating. The good agreement between the two conventional K-Ar dates for this sample (3.62 ± 0.09 Myr and 3.59 ± 0.05 Myr) and the $^{40}\text{Ar}/^{39}\text{Ar}$ date which yielded an isochron age of 3.55 Myr (Fig. 4) indicates that initial excess ^{40}Ar is not a problem with this sample, and that these dates give an average age for this horizon of 3.59 Myr.

Two dates were obtained from a biotite-bearing crystal-lithic tuff 60 cm thick lying approximately 50 m below tuff *c* near the middle of the thickest section of the Laetolil Beds at location

B (Fig. 2). The tuff is of nepheline composition, containing abundant augite and altered nepheline and 2–3% of biotite, some crystals of which are as much as 1 cm in diameter. Calcite cements the crystals and fragments together. Biotite was separated by crushing, screening and hand picking and was cleaned of calcite with dilute HCl. Two dates, 3.82 ± 0.16 Myr and 3.71 ± 0.12 Myr, average 3.77 and give a lower limit to the age of the hominid remains (which occur below but close to the dated xenolithic horizon higher in the section) in the 30 m section at locality *A* (Fig. 2) whose base projects approximately 20 m above this tuff at locality *B* (Fig. 3).

Fauna

The fossiliferous deposits in the Laetolil area have been subdivided, for purposes of collecting, into 26 localities. These subdivisions are based on existing topographic features such as grassy ridges, lines of trees, stream channels and so on. This has provided a means of dividing the fossiliferous area into units of restricted size, but does not relate to the former topography.

Eighteen of these localities are in the Garusi valley, one in the valley at the head of the Olduvai Side Gorge, one in the Gadjingero valley, five in a valley to the south of the Garusi river and one in an isolated position to the west (Fig 2).

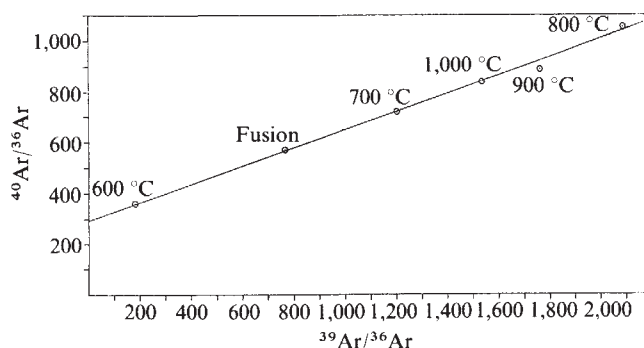
Identifiable fossils noted on the exposures were either collected and registered or listed on the sites. Specimens from the Laetolil Beds were distinctively cream coloured or white and sometimes chalky in texture, but the surface material also included brown, grey or black specimens, often rolled. These have been excluded from the material under review, together with fossils which have adhering matrix clearly dissimilar from the tuffs of the Laetolil Beds. Among these are all remains of *Hippopotamus*, *Equus*, *Theropithecus*, *Phacochoerus* and *Tragelaphus*, formerly included in the Laetolil fauna, with the exception of *Hippopotamus*.

The fossil material from the Laetolil Beds is dispersed and fragmentary and it is not possible to assess the number of individuals represented. In this article, 'numbers of fossils' refer to individual bones and teeth, except in the case of clear association, confined almost entirely to remains of *Serengetilagus* and *Pedetes*, some of which were associated and even articulated.

Table 2 shows the mean percentage frequency of the more common vertebrate groups at several of the richer localities. A total of 6,288 fossil specimens was identified from the localities considered here.

Reptiles are represented by snake vertebrae at three localities and by tortoises at all localities. The latter have an average frequency of 2.2% and include several giant specimens. Avian remains occur widely and at several localities birds' eggs were completely preserved. There is one example of a shattered clutch of at least eight eggs, rather smaller in size than eggs of domestic fowl. Primates were found at 15 localities, and in one area they constitute 3.8% of the fauna. Both cercopithecines and colobines are present (M. G. Leakey, personal communication).

Fig. 4 $^{40}\text{Ar}/^{39}\text{Ar}$ incremental heating of biotite from xenolithic horizon, locality *C*. Isochron age, 3.55 Myr; $^{40}\text{Ar}/^{39}\text{Ar}_0 = 294$; $^{40}\text{Ar}/^{39}\text{Ar} = 0.35318$; $J = 0.005204$.



Rodents are fairly well represented, although not abundant. Of the specimens identified by J.J. Jaeger, the most common are *Pedetes*, *Saccostomus* and *Hystrix*. The carnivore fauna is characterised by a high percentage of viverrids, constituting 32% of all the carnivore specimens. Large carnivores are represented by hyaenids, of which there are several genera, by felids and a machairodont.

Proboscidea include *Deinotherium* and *Loxodonta* sp. (M. Beden, personal communication). There is no evidence that the equid material (other than that derived from later deposits) includes any genus except *Hipparion*. The suids consist only of two genera, *Potamachoerus* and *Notochoerus* (J. Harris, personal communication). The presence of *Ancylotherium* and *Orycteropus*, noted in previous collections, is confirmed and the existence of two rhinocerotids has been established by the discovery of skulls of both *Ceratotherium* and *Diceros*, although only the former was listed previously.

Among the giraffids, *Sivatherium* and a small form of giraffe are equally common. *Giraffe jumae* is also present but is much

Table 2 Mean percentage frequency of bones of more common vertebrate groups

Group	Mean percentage	Range	Nos of localities
Bovidae	43.0	29.5–57.9	18
Lagomorpha	14.4	5.6–24.0	18
Giraffidae	11.2	6.8–23.2	18
Rhinocerotidae	9.7	5.7–17.3	18
Equidae	4.4	1.7–7.1	18
Suidae	3.6	1.0–7.1	18
Proboscidea	3.4	1.0–5.1	18
Rodentia	3.3	0.9–7.2	17
Carnivora	3.1	0.8–8.2	17

less well represented. The bovid fauna is chiefly characterised by the very high percentage of *Madoqua* (dikdik). In 18 localities dikdik range from 1.5 to 37.7% of all bovid specimens, with a mean percentage of 15.1%.

The 1975 field season, although mainly confined to surface collection, has established that previous collecting had sampled faunas of several time periods. It is now possible to exclude some genera from the published lists of fauna from the Laetolil Beds^{2,9}, such as *Theropithecus*, *Tragelaphus*, *Equus* and *Phacochorus*.

Fossil hominids

Thirteen new fossil hominid specimens were recovered from the Laetolil site during 1974 and 1975. The remains include a maxilla, mandibles and teeth. This sample displays a complex of characters seemingly demonstrative of phylogenetic affinity to the genus *Homo*, but also features some primitive traits concordant with its great age.

The hominid specimens are listed in Table 3. Provisional stratigraphic correlation and dating have placed the hominid remains as shown in Fig. 3. With the exception of Laetolil hominids (LH) 7 and 8, all specimens retain the matrix characteristic of the Laetolil Beds from which they have been weathered or excavated. There is no reason to doubt that all the specimens derive from the Laetolil Beds as reported.

The Laetolil hominid sample consists of teeth and mandibles. Important features of these specimens are described here, followed by a brief preliminary discussion regarding the phylogenetic status of the fossils.

Remains of deciduous and permanent dentitions have been recovered. The dentitions consist of two maxillary and four mandibular partial tooth rows. Compared with the rest of the East African Pliocene/early Pleistocene hominid sample, the Laetolil anterior teeth are large and the postcanine teeth of small to moderate size.

Deciduous dentition

Canines (LH 2, 3) Single upper and lower deciduous canines are known. The lower is a slightly projecting, sharp conical tooth in its damaged state. It is smaller but its overall morphology is similar to its permanent counterpart.

First molars (LH 2, 3) The upper first deciduous molar displays spatial dominance of the protocone and a well-marked mesio-buccal accessory cusp defined by a strong anterior fovea. The lower first deciduous molar is molarised, with four or five main cusps depending on hypoconulid expression. There is a spatially dominant protoconid with a large flat buccal face, a lingually facing anterior fovea, and an inferiorly projecting mesiobuccal enamel line. Vertical dominance of the protoconid and metaconid is marked in lateral view.

Second molars (LH 2, 3, 6) Upper and lower deciduous second molars take the basic form of the analogous permanent first molars but are smaller in overall size. The dM₂ of LH 2 is the only lower molar in the Laetolil sample bearing any indication of a tuberculum sextum.

Table 3 Hominid remains recovered in 1974–75

Laetolil hominid (LH)	Locality	Specimen consists of	Discovered by
1	1	RP ⁴ fragment	M. Muoka
2	3* ¹	Immature mandibular corpus with deciduous and permanent teeth	M. Muluila
3 (a–t)	7*	Isolated deciduous and permanent teeth, upper and lower	M. Muoka
4	7	Adult mandibular corpus with dentition	M. Muluila
5	8	Adult maxillary row: I ² to M ¹	M. Muluila
6 (a–e)	7*†	Isolated deciduous and permanent teeth, upper	M. Muoka
7	5	RM ¹ or ² fragment	M. Muoka
8	11	RM ² , RM ³	E. Kandindi
10	10W	Fragment left mandibular corpus with broken roots	E. Kandindi
11	10W	LM ¹ or ²	E. Kandindi
12	5	LM ² or ³ fragment	E. Kandindi
13	8	Fragment right mandibular corpus with broken roots	M. Jackes
14	19	Isolated permanent teeth, lower	E. Kandindi

LH 9 was not valid.

**In situ*.

†LH 3 and 6 associated in mixed state.

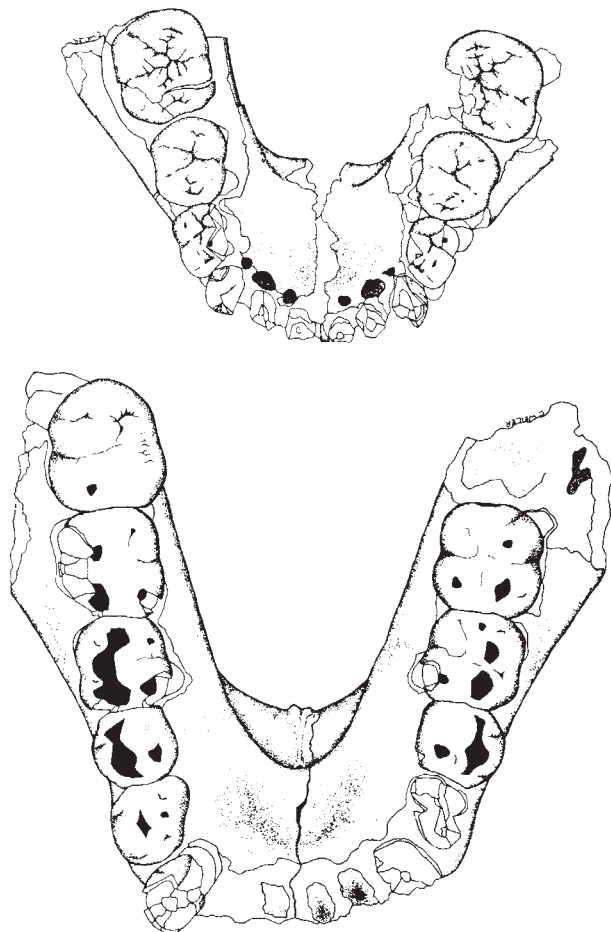


Fig. 5 Occlusal views of juvenile and adult mandibles (LH 2 and 4).

Permanent dentition

Incisors (LH 2, 3, 5, 6, 14) The single upper central incisor is very large and bears pronounced lingual relief. The upper lateral incisors are smaller, with variable lingual relief. The lower incisors are narrow and very tall, with minimal relief.

Canines (LH 2, 3, 4, 5, 6, 14) The incompletely-developed upper canine LH 3 is large, stout and pointed, bearing pronounced lingual relief. LH 6 is slightly smaller, with less lingual relief and a tall, pointed crown. LH 5 bears an elongate dentine exposure on its distal occlusal edge but does not project beyond the occlusal row in its worn state. The lower canines are similar to the uppers in their great size, height and lingual relief.

Premolars (LH 1, 2, 3, 4, 5, 6, 14) The upper premolars tend to be buccolingually elongate and bicuspid, with the lingual cusp placed mesially and an indented mesial crown face. The two lower third premolars each have a dominant buccal cusp with mesial and distal occlusal ridges, and a weak lingual cusp placed mesial of the major crown axis. The long axis of the oval crown crosses the dental arcade contour from mesiobuccal to distolingual, donating a 'skewed' occlusal profile to the tooth. The lower fourth premolars are fairly square in shape with major buccal and lingual cusps and moderate talonids.

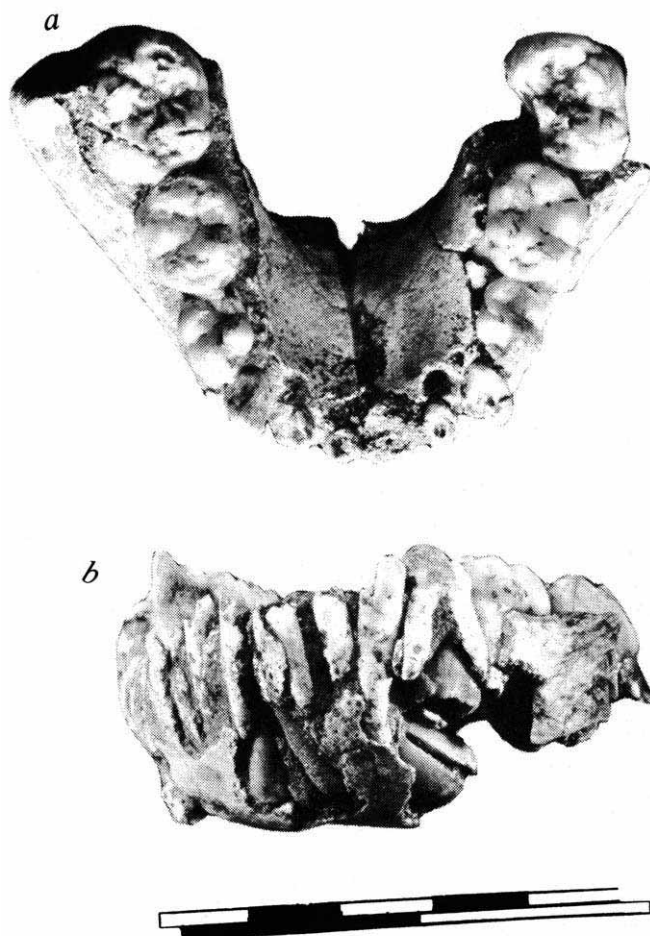
Molars (LH 2, 3, 4, 5, 6, 7, 8, 11, 12, 14) The upper molars are of moderate size, their relative proportions unknown. They show a basic four-cusp pattern with spatial dominance of the protocone and typical expression of a pit-like Carabelli feature. The lower molars display progressive size increase from first to third in LH4. They have a fairly square occlusal outline with hypoconulid appressed anteriorly between hypoconid and entoconid and no trace of a tuberculum sextum. The Y-5 pattern of primary fissuration is constant.

Mandibles

Juvenile mandible (LH 2) This specimen (Figs 5 and 6) is incompletely fused at the midline, and the developing crowns of the permanent canines and premolars are exposed in the broken corpus. The first molars have just reached the occlusal plane. Only the posterior aspect of the symphysis shows fairly intact contours, with a concave post-incisive planum and incipient superior transverse torus. The genioglossal fossa is obscured by midline breakage. Associated distortion has artificially increased the bimolar distances.

Adult mandible (LH 4) The adult mandibular corpus is well preserved, with the rami missing (Figs 5 and 7). The dental arcade is essentially undistorted, and presents fairly straight sides which converge anteriorly. The anterior dentition has suffered *post mortem* damage and loss, except for the right lateral incisor which seems to have been lost in life. Largely resorptive alveolar pathology has obliterated its alveolus and has affected the adjacent teeth. There is development of wide interproximal facets for the canine teeth but no C/P₃ contact facet. This combines with observation of extensive wear on the buccal P₃ face to suggest that C/P₃ interlock has prevented mesial drift from eliminating the C/P₃ diastema. Judging from the preserved posterior incisor alveoli, these teeth were set in an evenly rounded arcade, projecting moderately anterior to the bicanine axis. The internal mandibular contour is a very narrow

Fig. 6 Juvenile mandible from the Lactolil Beds (LH 2). *a*, Occlusal view; except for the first molars all the teeth are deciduous. *b*, Front view showing the central incisors, canine and P₃ in the bone.



parabola in contrast to the wider basal contour which displays great lateral eversion posteriorly. There are weak to moderate superior and inferior transverse tori, the latter bearing strong mental spines.

The anterior root of the ramus is broken at its origin, lateral to M_2 . Occlusal and basal margins diverge strongly anteriorly, resulting in a deep symphysis. The symphysis is angled sharply posteriorly and the anterior symphyseal contour is rounded and bulbous. The lateral aspects of the corpus have very flat posterior portions and distinctive hollowing in the areas above the mental foramina at the P_3 to P_4 position. The corpus is tall and fairly narrow, especially in its anterior portion.

Implications of the specimens

The Laetolil fossil hominid sample, including the original Garusi maxillary fragment⁸, seems to be representative of only one phylogenetic entity or lineage. The variations observed in the material seem to be primarily size-based and stem from individual and sexual factors.



Fig. 7 Adult mandible from the Laetolil Beds (LH 2), occlusal view.

The deciduous teeth, particularly the lower deciduous first molars, display remarkable similarity to hominid specimens from South Africa (Taung; STS 24)¹⁰ as well as to individuals tentatively assigned to *Homo* in East Africa (KNM ER 820, 1507)^{11,12}. They depart strongly from the pattern of molarisation displayed in the South African "robust" specimens (TM 1601; SK 61, 64)¹⁰ as well as from East African specimens generally assigned to the same hominid lineage (KNM ER 1477)¹³.

The Laetolil permanent canines and incisors are relatively and absolutely large and bear a great deal of lingual relief. They ally themselves similarly to earlier hominid specimens such as STS 3, 50, 51, 52; MLD 11; OH 7, 16; KNM ER 803, 1590 (refs 10, 15, 18–21), as well as to the younger African and Asian specimens usually assigned to *Homo erectus*. These features set the Laetolil specimens apart from the sample including SK 23, 48, 876 and so on; Peninj; KNM CH 1; OH 5,

38; KNM ER 729, 1171 (refs 10, 15, 18–21). The Laetolil permanent premolars show none of the molarisation seen in the latter specimens and bear particularly strong resemblances to South and East African material (STS 51, 52, 55; OH 7, 16, 24; KNM ER 808, 992 (refs 10–12, 14)).

The Laetolil permanent molars are consistent in aligning with the South African "gracile" australopithecus and the East African *Homo* material in both size and morphology. The molars do not display the increased size, extra cusps or bulging, expanded, "puffy" development of the individual cusps seen in high frequency among South and East African "robust" forms (SK 6, 13, 48, 52; TM 1517; Peninj; KNM CH 1; KNM ER 729, 801, 802) (refs 10, 17–20). The adult mandible has resemblances to certain East African specimens such as KNM ER 1802, with similar corpus section and basal eversion.

The Laetolil fossil hominids have several features possibly consistent with their radiometric age. These traits include the large crown size and lingual morphology of the permanent canines; the morphology and wear of the C/P₃ complex; the buccolingually elongate upper premolars; the overall square occlusal aspect of the lower permanent molars; the low symphyseal angle; the bulbous anterior symphysis; the relatively straight posterior tooth rows; the low placement of the mental foramina, and the distinctive lateral corpus contours including small, superiorly placed extramolar sulci.

Preliminary assessment indicates strong resemblance between the Laetolil hominids and later radiometrically-dated specimens assigned to the genus *Homo* in East Africa. Such assessment suggests placement of the Laetolil specimens among the earliest firmly dated members of this genus. It should come as no surprise that the earlier members of the genus *Homo* display an increasing frequency of features generally interpreted as "primitive" or "pongoid like", which indicate derivation from as yet largely hypothetical ancestors.

Much of the recently discovered comparable fossil hominid material from the Hadar region of Ethiopia shows strong similarity to the Laetolil specimens²³, and further collection combined with detailed comparative analysis of material from both localities is essential for the further understanding of human origins. The Laetolil collection adds to the developing phylogenetic perspective of the early Hominidae and emphasises the need for taxonomic schemes reflective of and consistent with the evolutionary processes involved in the origin and radiation of this family.

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Non-genetic individuality: chance in the single cell

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The individuality of bacterial cells grown in homogeneous conditions was demonstrated by showing characteristic behavioural differences which persist over their lifespans. Poissonian fluctuation of small numbers of generator molecules can explain this individuality and may apply to such processes as differentiation and asynchrony of cultures.

BIOLOGICAL systems are constantly confronted with chance occurrences in their environmental conditions and internal processes. An elaborate biological apparatus has evolved to utilise chance in genetics for the survival of the species. Some feedback mechanisms, on the other hand, are designed to insulate the individual against chance fluctuations of environmental conditions. How much influence has chance in internal cellular processes in determining the metabolic state and behaviour of the single cell? More specifically, if cells containing identical chromosomes could be grown in environmentally homogeneous conditions, how similar would they be in their biochemical characteristics?

Uniform growth conditions can be obtained with agitated suspension cultures, since the spatial inhomogeneities which inevitably develop in stationary surface growth (for example, tissue culture plates or agar surfaces) are continuously randomised in swirled liquid culture. The problem of apparently non-genetic variation of cells in such a homogeneous environment has arisen in studies of several phenomena. For example, bacteriophage burst sizes¹, cell division times², bacterial flagellar phases^{3,4} and β -galactosidase concentrations⁵⁻⁷ all show variation difficult to attribute to environmental inhomogeneities or genetic causes. The question of individuality is difficult to pursue in these cases because one needs measures of characteristics of individual cells which can be ascertained essentially instantaneously and which do not perturb the cell's biochemical state. With such instantaneous measures an individual cell can be compared with itself at various stages of its life cycle and with other individual cells in an isogenic population. Such

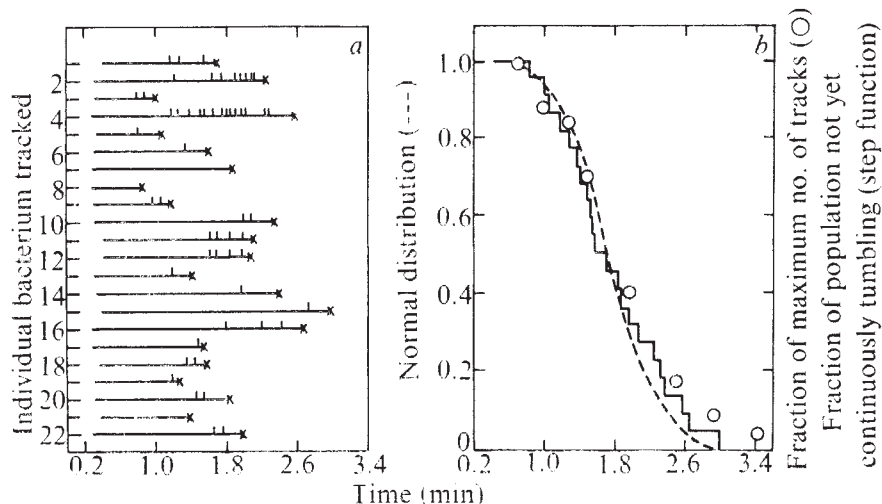
measures became available in our studies on chemotaxis and enabled us to approach the question of individuality in bacteria as an illustration.

The chemotactic migration of bacteria can be studied quantitatively. In the absence of chemotactic stimuli a bacterium shows brief periods of swimming in an approximately straight line (smooth swimming), interrupted by a rapid irregular motion, which reorients its swimming direction (tumbling). Tumbling is suppressed when the bacterium swims in a favourable direction in a chemical gradient and this regulation of tumbling frequency results in net migration up gradients of attractants and down gradients of repellents⁸⁻¹⁰. The modulation of tumbling frequency is caused by temporal changes in attractant or repellent concentration which are analysed by the sensing system and transmitted to the flagella^{8,11}. For example, if the concentration of an attractant in the bacterial environment is suddenly increased, this temporal gradient transiently suppresses tumbling in the bacteria. Although the details of the temporal gradient sensing mechanism are unknown, control of tumbling can be rationalised as caused by changes in the levels of a tumble regulator^{8,12}. The concentration of this tumble regulator is thus a key property of the cell since it provides an important survival mechanism for the bacterium. Its properties can be measured quantitatively in two ways: (1) in the sensitivity to stimuli as measured by recovery from temporal gradients^{12,13} and (2) in the tumbling frequency when there is no gradient^{8,12} as an indicator of the steady-state level of tumble regulator. These two properties were studied for individual bacteria as a test for non-genetic variability.

Sensitivity to gradients of attractant

Our first study of individual bacteria involved subjecting a bacterial clone of a tumbly mutant strain to a temporal gradient of serine, using the sudden mixing methods described earlier^{8,13}. The transient smooth swimming in response to a temporal gradient stimulus is especially easy to quantify with tumbly mutants. The individual bacteria were kept in focus by rough tracking through a movable microscope stage which keeps the

Fig. 1 Tumbly mutant recovery individuality. All bacteria were grown in homogeneous conditions as described previously¹³. In *a* the length of each line represents the time interval over which an individual bacterium was tracked. For each of the 22 lines the temporal gradient stimulus 0→0.5 mM L-serine was delivered to ST171 at time 0 (as previously described¹³) and the bacterium nearest the centre was followed until it tumbled continuously for 30 s. Times at which the bacterium briefly tumbles are represented as $\perp$, and the time at which it began to continuously tumble is represented as $\times$. From the data in (*a*) the fraction of the bacteria not yet continuously tumbling was plotted against time to yield the step function in (*b*). $\bigcirc$, Tumble frequency assay¹³ results for ST171 subjected to the same stimulus; ---, a cumulative normal distribution with the same mean and variance as the data obtained from tracking.



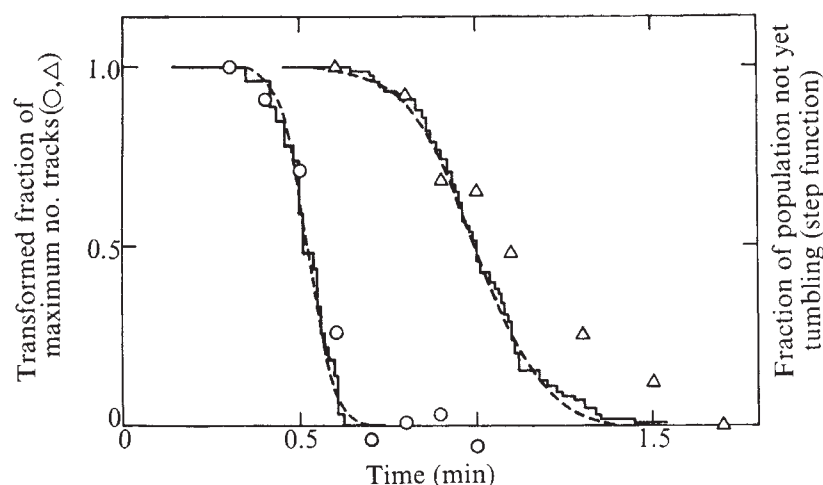


Fig. 2 Wild-type recovery individuality. Points are tumble frequency assay¹³ results transformed by equation (1) (see text) for ST1 subjected at time 0 to the temporal gradient stimuli 0→0.01 mM (O) l-aspartate and 0→1.0 mM l-aspartate (Δ). Step functions are cumulative times to the first tumble after stimulation for these two same stimuli derived from tracking as described in Fig. 1. The dotted lines are cumulative normal distributions with the same means and variances as the two sets of tracking data.

bacterium in the centre of the field. A videotape recording could be played back in slow motion to describe in detail the behaviour over a long period. The results for 22 individuals of a constantly tumbling mutant subjected to a serine stimulus are shown in Fig. 1a. There is great variability in the time that individual bacteria take to return to their prestimulus tumbling pattern. Data of the same type (Fig. 2) were obtained for a wild-type strain which also swims smoothly immediately after stimulation and returns to intermittent tumbling (its normal non-gradient state). If data from tracking are plotted on the same graph as a recovery curve for a population subjected to the same stimulus in the tumble frequency assay¹³, the curves are superimposable (Fig. 1b).

The results of Fig. 1b mean that the form of the recovery of a population from a stimulus is caused by the variability of the individuals in the population. In fact, if we define $F(t)$ as the fraction of a population which has recovered at time t after delivery of a stimulus and $S(t)$ as the probability of producing a smooth swimming track, equation (1) describes the behaviour of the bacteria. S_∞ is zero for the constantly tumbling mutant and has a finite value, greater than zero, for wild-type bacteria.

$$1 - F(t) = \frac{S(t) - S_\infty}{1 - S_\infty} \quad (1)$$

The bacteria used in these experiments were at most 30 generations away from a single cell parent. An extremely high spontaneous mutation rate would have to be postulated to explain our data in terms of genetic variation, as the following calculation shows. Where m is the probability of occurrence of any mutation affecting chemotaxis per generation, after 30 generations the probability of a bacterium not containing a mutation is $(1 - m)^{30}$. Therefore for at least 50% of the bacteria to have acquired one or more mutations affecting chemotaxis,

$$(1 - m)^{30} < 0.5$$

or

$$m > 2.3 \times 10^{-2}$$

There are only about 10 genes specifically involved in *Salmonella* chemotaxis¹⁴, but even the liberal estimate that a mutation in any one of 100 loci could alter chemotactic behaviour requires that the mutation rate per locus must be greater than 2×10^{-4} , which is at least 10^3 times higher than usual spontaneous mutation rates. Therefore we conclude that (1) there is a large non-genetic individual variability in the response of both tumbling mutants and wild-type bacteria to stimuli and (2) the results of the tumble frequency assay are a direct measurement of this variability.

Individuality and phase of cell cycle

The experiments described above establish that the cell types present do not show identical behaviour, but do not distinguish between the alternatives: (1) that the individuality is created by one or more random events in the history of the bacterium or (2) the cell has no intrinsic individuality but its properties vary according to its position in the cell division cycle. In the latter case the asynchrony of the culture would give the appearance of individuality where it does not in fact exist. To distinguish between these alternatives, the response of the bacteria were compared as a function of cell length. Cell length is known to be correlated with position in the division cycle¹⁵ and bacterial lengths are clearly visible in the measurements from the tumble frequency assay. The lengths of smooth swimming tumbly mutant bacteria following stimuli at a time at which 95% of the bacteria were smooth swimming were compared with the same values when less than 10% of the bacteria were still smooth swimming. The former gives values for the overall population and the latter for the longest responding 10% of the bacteria. The distributions of the bacterial lengths are shown in Table 1. There is no significant shift in the distribution of the lengths as recovery proceeds and therefore the first alternative must be correct.

Repeated stimulation of a bacterium

If the different responses of isogenic bacteria are not related to the phase of the cell cycle, it can then be asked whether they (1) respond differently because of chance events during or

Table 1 Independence of recovery times and position in the cell division cycle

Body length (μm)	Fraction of population of body length indicated with > 95% smooth swimming	Fraction of population of body length indicated with < 10% smooth swimming
< 1.33	0.00	0.00
1.33–2.00	0.05	0.05
2.00–2.67	0.24	0.21
2.67–3.33	0.32	0.33
3.33–4.00	0.26	0.28
4.00–4.67	0.09	0.10
4.67–5.33	0.04	0.03
> 5.33	0.00	0.00

The lengths of ST171 bacterial bodies were measured on projected films from the tumble frequency assay¹³. 186 individual bacterial lengths were measured from photographs at times for which less than 5% of the bacteria had recovered from the serine stimuli to define the distribution designated >95% smooth swimming. 212 individual bacterial lengths were measured after more than 90% had recovered to establish the distribution in the last column.

Table 2 Response times (s) of individual bacteria on repetitive stimulation in growth conditions

Bacterium	A	B	C	D	E
Stimulus time (min)					
15	237	224	190	165	136
30	254	164	126	176	134
60	> 300	265	191	136	145
75	> 300	248	217	144	143
90	> 300	253	172	152	148*
Average clockwise (smooth) response (s)	> 278	231 ± 18	179 ± 15	155 ± 7	141 ± 3

Bacteria of strain SL3625, a leaky *fla AIII* mutant which produces small numbers of flagella, were tethered with flagella antibody to a coverslip by a modification of the procedure of Silverman and Simon¹⁶. Bacteria were grown as described¹³ except 200 µM *p*-hydroxybenzoic acid (*p*HBA) was added to the growth medium to improve motility (unpublished results of J. Bar-tana, B. Howlett and D.E.K.). The coverslip with attached bacteria was used to cover an open temporal gradient observation cell of the type described by Macnab and Koshland⁸. The observation cell was previously filled with the wash medium to allow a liquid seal to form immediately the coverslip was put in place (time 0 min). The behaviour of bacteria in one microscopic field at × 500 magnification was recorded on videotape as follows. Cells were washed extensively (5 min at 12.7 chamber volumes per min) with the growth medium (without *p*HBA); at the times indicated the output line to the observation cell was switched to a line containing this same medium + 10 mM α -methylaspartate and a 15-s pulse of 12.7 chamber volumes of attractant was delivered. The resulting immediate long clockwise rotation interval of an individual bacterium was measured as its "clockwise response". After 5 min, the line was switched back to medium not containing the attractant, and a 1-min pulse of 12.7 chamber volumes per minute delivered. Each bacterium in the field which was rotating for all stimuli was analysed by repetitive playback of the videotape. The doubling time of SL3625 in this medium is 112 min. Mean responses are reported ± 1 s.e.m.

*Bacterium E divided 20 s after the delivery of the stimulus at 90 min.

immediately preceding the time of the measurements, that is they are long responders at one time but short responders later, or (2) whether an individual bacterium is committed for an extended period to a certain type of response, that is some bacteria are short responders throughout their lifespan. To test the response of an individual bacterium over its cell division cycle, individual bacteria were tethered to the surface of microscope coverslips by antibodies to individual flagella, as described before¹⁶. Using this tethering technique, Berg and Tedesco¹⁷ have shown that individual bacteria can be examined over extended periods and that tethered cells differ in their chemotactic behaviour. The tethering method permits attractant to be delivered and washed out at various intervals which are minutes or even hours apart and the response of the same individual bacterium recorded over the entire time span using videotape. Larsen *et al.*¹⁸ established that counterclockwise rotation and clockwise rotation of a tethered cell (observed along the axis from the flagellum to the body) are equivalent to the tumbling and smooth swimming, respectively, of a free swimming cell.

Table 2 shows the results of repetitive stimulation of five tethered bacteria in the same microscopic field with the attractant α -methylaspartate. The attractant was delivered five times and removed completely between stimuli. The delivery of large stimuli of attractant suppressed counterclockwise (tumbling mode) rotation in each bacterium and caused a uniform clockwise (smooth swimming mode) rotation for several minutes with eventual recovery to intermittent clockwise and anticlockwise rotation. All bacteria were observed to increase to nearly double their mass during the measurements, yet the data show that their individual sensitivities to the stimulus were maintained. The same experiment was performed with six bacteria in a non-growth medium and these bacteria also maintained their tendencies to be either long or short responders to α -methylaspartate¹⁹. Thus, whether growing or not, individuals retain characteristically different sensitivity to stimuli for extended periods and the cells are not continuously randomising with respect to their chemotactic behaviour.

Relationship of unstimulated to stimulus-induced behaviour

If the bacteria differ in their characteristic recovery times, are they also different in some other feature of their sensory system? The unstimulated behaviour of tethered cells consists of alternating clockwise and anticlockwise rotation intervals with each of the two rotation modes terminated by Poissonian (random) processes¹⁷. The patterns of individual cells showed that the mean clockwise and counterclockwise intervals were not identical for all bacteria. In view of the randomness in lengths of clockwise and counterclockwise intervals for any individual bacterium, the theoretical standard error of the mean was calculated to compare with the empirical s.e.m. obtained from actual measurement of the clockwise and counterclockwise intervals (Table 3). The good agreement between theoretical and empirical values is assurance that the amount of data collected to determine the intervals is sufficient.

On comparison of these spontaneous rotation intervals with the response to stimuli (Table 3) an excellent correlation was observed. The size of the clockwise response to an α -methylaspartate stimulus for an individual bacterium depends nearly linearly on its mean clockwise (smooth swimming mode) interval in non-stimulus conditions as shown in Fig. 3. The size of the anticlockwise interval (tumbly mode) does not seem to influence the stimulus behaviour.

To check this in another way, 82 free swimming cells were examined both for the time to the first tumble after temporal gradient stimulation with aspartate and for the mean time between tumbles for 1 min after the first tumble. Although this measurement is less precise than measurements with tethered cells (because a particular individual can be observed for only one stimulation and for only a short time after the stimulus), we found a correlation coefficient of 0.5 between the average length of a run and the recovery time.

These data provide a correlation between the steady-state tumbling pattern of individual bacteria and their sensitivity and recovery from stimuli. The steady-state pattern of tumbling,

Table 3 Unstimulated behaviour and smooth responses of individual bacteria

Bacterium	F	G	H	I	J
No. of intervals	154	84	186	134	82
Mean clockwise (smooth rotation mode) length (s)	2.45	1.93	1.40	1.54	2.97
Measured s.e.m.	0.28	0.26	0.16	0.19	0.54
Calculated s.e.m.	0.28	0.30	0.15	0.19	0.46
Mean anticlockwise (tumbly rotation mode) length (s)	0.88	1.69	0.80	1.63	0.70
Measured s.e.m.	0.12	0.25	0.08	0.22	0.16
Calculated s.e.m.	0.10	0.26	0.08	0.20	0.11
Mean clockwise (smooth) response (s):	211 ± 10	152 ± 6	120 ± 8	123 ± 6	247 ± 7

The experiment was performed by a procedure identical to that of Table 2 except: (1) instead of growth medium cells were washed with Vogel Bonner salts + 10⁻⁵M EDTA; (2) attractant (10 mM α -methyl aspartate) was prepared in Vogel Bonner salts + 10⁻⁵M EDTA; (3) after the initial wash of 5 min of 12.7 chamber volumes per min, a continuous flow rate of 1.6 chamber volumes per min was maintained throughout the experiment; (4) at 5 min intervals the input line was switched between attractant and buffer solutions to give a total of six repetitive stimuli. About 2 min of video recordings of the bacteria in periods immediately preceding delivery of attractant were examined in slow motion (sevenfold) replays. Keyboard inputs to a Data Span 500 paper tape punch with timing circuitry²⁰ were punched when the bacterium being analysed reversed rotation direction for at least 1/4 of a full turn. The paper tape containing the reversal information and the automatically entered times of keyboard entries was processed by computer. The calculated s.e.m. was determined with the assumptions that (1) the measured mean is the true mean and (2) both clockwise and anticlockwise intervals are distributed exponentially¹⁷. Clockwise responses are reported as the mean ± 1 s.e.m.

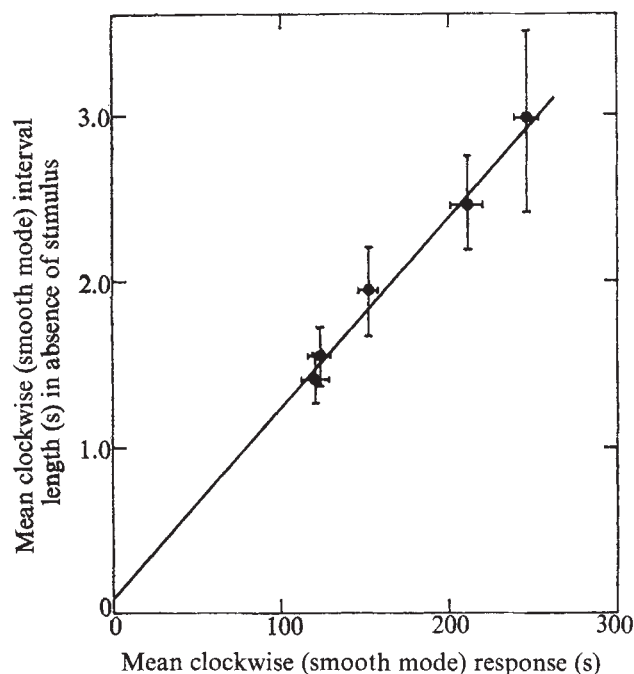


Fig. 3 Unstimulated clockwise (smooth mode) rotation related to stimulus induced clockwise (smooth mode) rotation. The prestimulus clockwise (CW) mean interval length and the mean CW response were taken from Table 3. Error bars represent $\pm$ one standard error of the mean. The line is a least squares fit to the data.

therefore, is also a feature of the bacterial individuality and is retained throughout the generation time of the bacterium.

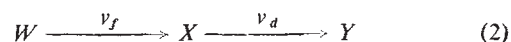
Implications of individuality

Study of individual bacteria in various ways has established that a bacterial culture which is genetically homogeneous and grown in homogeneous nutrient conditions nevertheless produces characteristically different individuals. These individuals retain their properties throughout their life cycle, showing that the individuality is not a momentary effect of random chance bombardments by a fluctuating environment. Moreover, the individuality is not a function of position in the cell division cycle.

How could such individuality be produced? A bacterium is a small cell. In the process of division and replication of the cellular components there may well be certain molecules which are present in such low amounts that they are subject to Poissonian variation. In general, the standard deviation of a Poisson distribution is the square root of n , where n is the mean number of events. If there are 10^4 molecules of a particular type, the s.d. would be 100 molecules, giving a 1% deviation. If, on the other hand, only 100 molecules are produced, a 10% s.d. would arise. In many cellular processes less than 100 molecules participate (for example, there are only 10–20 molecules of *lac* repressor per cell²⁰ and enzyme cofactor concentrations are extremely low²¹). In other processes there may be more total molecules but their ultimate number may have been determined by a small number of generating molecules (for example, there are only 6–14 *trp* mRNA molecules per bacterial cell, and each mRNA molecule is translated an average of 20 times²²). Probabilistic fluctuations in small numbers of molecules have been discussed as possible causes of bacteriophage burst size variation by Delbrück¹ and bacterial β -galactosidase concentration variation by several investigators^{5–7}.

In the case of chemotaxis, it has been postulated that the tumble regulator is produced and destroyed according to scheme 2, implying that some enzymes or structural molecules control the velocities of the steps represented by v_f and v_d . If a

small number of mRNA molecules code for the enzyme which, for example, controls the step v_d , Poissonian fluctuation in the mRNA concentration would generate quite different total numbers of molecules of this enzyme in different individuals. If this affects not only the steady-state tumbling pattern, but also the rate of recovery from a stimulus¹², both properties would be different from individual to individual over the lifespan of the cells. Conversely, if a particular cellular component were produced in a much larger number of copies



where X = tumble regulator over a much longer interval, Poissonian variation would be relatively small and one would not expect much variation in this property from cell to cell. The number of copies of an individual cellular component, at some crucial period in the life of the cell, would therefore determine whether or not that component would contribute to the non-genetic variability of the population.

The existence of such variability leads one to ask whether it has some advantage to the organism or whether it is a disadvantage which must be surmounted in the development of the species. Although there may be no significant selective pressure against non-genetic variability, one can certainly make an argument that non-genetic variability aids in the survival of a population subjected to widely varying conditions during its lifetime. If the bacterium has a single or monolithic reaction to a particular chemical gradient, it might migrate as a colony into a toxic situation or fail as a colony to be sufficiently sensitive to an essential new nutrient. If there were individual bacterial variation in a genetically homogeneous population, a few fractions of the bacteria might either be supersensitive or insensitive. These bacteria would normally not survive as effectively as the bulk of the more average optimised population. They would then have lower survival probabilities in most circumstances, an unimportant loss if the main body of the colony survived. If, on the other hand, the toxic and lethal situations described above arose, a small fraction, on the wings of the probability distribution, might constitute the only bacteria to survive and would then reproduce the species for future generations. Genetic variation would not accomplish the same purpose, since selection in a rare toxic situation would produce a mutant poorly adapted to the more common conditions of the environment. The population, however, was selected over evolutionary time for survival in all of the widely varying conditions of the environment. Thus non-genetic variability would be a preferred mechanism for accommodation to random fluctuations in the environment and genetic variability the preferred mechanism for accommodation to long lasting environmental changes. Induction and repression of enzyme synthesis reflect intermediate accommodations to fluctuations of a lengthy but impermanent type.

It would be intriguing if certain properties were found to be insulated against chance by the large number of copies in the cell at all crucial stages and other properties deliberately to originate in a small number of starting molecules, to create non-genetic diversity. An interesting example may arise in the differentiation of individual *Dictyostelium* amoebae into multicellular fruiting bodies. This development depends on the existence of a small number of early cyclic AMP-emitting founder cells which are believed to be genetically identical to the other cells in the population which are not early emitters²³. The difference between a founder cell and other cells in the culture seems to be the result of molecular accidents in the cell's individual history and this could be explained by a Poissonian distribution of properties deriving from small numbers of a cellular component as discussed here. Possibly over evolutionary time, larger cells and larger individuals were selected for decreased non-genetic variability; for example, identical human twins are morphologically much more similar than twin bacteria, and in higher species the genetic variability

carries the main load of ensuring the survival of the species. Poissonian variation at early stages in differentiation may be an advantage for the species even though ultimately chance is eliminated because of large numbers of cells developed in the individual.

Poissonian variation may be the reason for the rapid asynchronisation of synchronised prokaryotic²¹ and eukaryotic²⁵ cell cultures. If one or more steps leading to growth and cell division are controlled by a small number of molecules, then a Poissonian distribution should exist and cell cycles would quickly get out of phase. In fact there is evidence for individuality in bacterial division times² of the type we observe in chemotactic behaviour. Also in eukaryotic cells synchrony is not maintained even in populations selected for genetic homogeneity²⁵. To explain this, Smith and Martin²⁶ have proposed that growth of cultured cells is dependent on a random event occurring with a probability which is constant for all individual cells in postulated "indeterminate" phase in G_1 (the "A-state"). As evidence for this hypothesis they present generation time distributions which are exponential over most of their range. A chance external event could explain the probabilistic transition out of the A-state, if the Smith and Martin model is correct. Alternatively, as suggested here, the number of generating molecules for an enzyme required for passage through G_1 could be so small that Poissonian fluctuations result in a broad distribution of concentrations of this enzyme among the cells. Such a non-genetic individuality would be consistent with rapid asynchronisation, correlation of sibling generation times^{2,27,28} (if the enzyme half life is longer than one generation time), and the exponential character of the distributions of Smith and Martin. If Poissonian variation determines rates of passage through G_1 , or any stage in the cell cycle, cell synchrony over extended periods will be difficult to attain. If our explanation is correct, a possible approach to obtaining a synchronous cell line would be genetic manipulation, for example by episomes, to increase significantly the concentrations of the rate limiting enzymes.

Biological systems therefore can produce individual non-genetic diversity through a few generating molecules which determine a property at some key stages of development or can ensure against such chance diversity by increasing the number of generating molecules to a size which eliminates significant internal variation.

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letters to nature

Observations of X-ray and γ bursts

WE report on some bursts of hard X rays seen in March 1976 by the Ariel-V scintillation telescope (ST). The timescale of a few seconds, the correlation with low energy events seen by a proportional counter on the same satellite (ref. 1 and S. J. Bell-Burnell, private communication) and a photon energy > 50 keV, are factors which suggest a connection in origin between the gamma bursts of Klebesadel *et al.*² and the X-ray events noticed by Grindlay *et al.*³. A delayed arrival at high energies seems to be an important feature of our observations.

The Ariel-V scintillation telescope (area 8 cm^2 , 11° effective FWHM field of view) observed in a direction centred on RA 17 h 27 min, dec. -33.5° (1950) during March 8–11 with a broad energy channel extending from 50 to 190 keV. Counts from 0.5-s intervals were stored sequentially in sets (bins) of 16 locations which were scanned four times before proceeding to the next set. Each bin thus contained the superposition of 4 time profiles, 8 s long. An identical data mode was used for the 2–7-keV collimated proportional counter (CPC) of ref. 1. Burst sources MXB 1730–335, MXB1728–34, MXB1743–293 and MXB1742–297 were in the field of view of the ST but only the first two could be seen by the CPC. MXB1730–335 is the repetitive burster with small events as close as 7 s apart, while the others give at the most ~ 5 bursts d^{-1} (ref. 4).

Four very important proportional counter bursts were selected corresponding to those times when the ST data was expected to be free of all spurious events (events A, C, E, F Table 1), two of which (E, F) are plotted in Fig. 1, together with data from the ST. Absolute time is known to ± 30 s and this is shown at the beginning of each 32-s bin. In two cases the CPC data showed a pulse at least 8 s long; the double peaks probably arise from two successive scans. In at least 3 cases, the ST increase appeared delayed with respect to the CPC data. Because of the superposition of scans the delay cannot be determined uniquely. Table 1 lists the limits within which the delays lie, the significance in s. d. (σ) of the ST events, the pulse durations and the total energy in erg cm^{-2} assuming all high energy photons were at 50 keV.

In the case of events A and F, the larger of the two figures for minimum delay is more likely; the smaller figure results from taking smaller, secondary peaks in the CPC data as the associated events. Note the tendency for the high energy channel to carry at least as much energy as the low energy channel. Suggestions have been made for 1.39- and 1.36-h periodicities in the intervals between some bursts from this region (ref. 1 and Carpenter *et al.*, to be published). Only event A fits such a scheme (with a 1.36-h period).

To relate our work to searches for γ bursts, a scan of all the suitable March 8–11 data was made to pick out statistically

Table 1 Data from scintillation telescope and collimated proportional counter on Ariel V

Event	Time at start of bin (day in 1976 March)	2-7-keV channel		50-190-keV channel		Significance (σ)	Delay (s)	
		Width (s)	erg cm ⁻²	Width (s)	erg cm ⁻²		Min	Max
A	8.7765	8-32	2×10^{-7}	2.5	2.4×10^{-7}	3.7	11.5 or 1.5	59.5
B	8.9544	4	2.3×10^{-8}	3.5	4.1×10^{-7}	5.4	2.5	26.5
C	9.1656	8-32	1.8×10^{-7}	3.0	2.2×10^{-7}	3.3	0	28.0
D	9.4869	3	2.5×10^{-8}	6.5	4.3×10^{-7}	6	3.5	27.5
E	10.9419	4.5	5.2×10^{-8}	2.5	2.7×10^{-7}	5	3-4	34.3
F	11.0819	3.2	1.4×10^{-7}	1.6	2.9×10^{-7}	6.5	7.2 or 3.8	37.2

significant increases in 32-s intervals. Five such events were identified, corresponding to a rate of one every two hours with a significance such that the total chance probability of getting such increases in the data set was 10^{-6} . Two were events previously selected (A, F) and a further two, B and D, are added to Table 1 (B is also shown in Fig. 1) with correlated CPC data where any increase can only be in the 3-4 σ region. To compare with previous γ -burst measurements we assume the typical burst spectrum of ref. 5, integrate all the energy > 25 keV and correct the rate for the telescope solid angle by assuming (a) the sources are isotropic and (b) the sources are distributed according to the density of mean galactic matter. These two rates are shown in Fig. 2, along with Vela, Imp-7 and the balloon data of Bewick *et al.*⁶. The balloon search only included the galactic disk between longitudes 70°-230° within its field of view. It is clear that at some times at least, 'X-ray' bursts, which could equally well be classified as small ' γ bursts' in the 10^{-7} - 10^{-6} erg cm⁻² energy range, are far more likely to be seen from the certain regions in the galactic disk than from elsewhere, a conclusion already suggested by Bewick *et al.*⁶. We

have insufficient data at present, however, to compare the results with the prediction of a globular cluster origin (compare with ref. 3). Although we cannot measure the energy spectrum with Ariel V, one notes that Grindlay *et al.*³ found an exponential fit to their data with kT hardening from 5-20 keV. Thus it is possible to regard γ bursts as being particularly 'hot' X-ray bursts. Since, however, Lewin *et al.* saw no apparent hardening with time of bursts from MXB1730-335, it is clear that there is more than one source mechanism, or variant of the mechanism, at work. Also our events may not arise from this repetitive burster.

The importance of the spectral hardening with time has already been discussed by Grindlay *et al.*³ who saw kT increase to 20 keV, but we have shown that at late times and for some events, the energy can reside almost entirely in > 7-keV photons. Their suggested mechanism, involving Inverse Compton acceleration of an initial low energy pulse, requires an optical depth > 1 and is unlikely to fit the acceleration (by a factor > 10) required by our observations, which consequently greatly increases the optical depth necessary. Let us instead think in terms of variants of a mechanism in which gravitational energy is transformed by a shock to an optically thick plasma emitting by a thermal process. Suppose at first that the shock is travelling between a companion star and a compact object, mass M , by near free fall in the gravitational field in a comparatively rarified medium, from an initial position $R_{9,0}$ (units of 10^9 cm). Individual particles behind the strong shock can reach a thermal energy $E \leq (1/4)^{1/2} m_p U^2$ if the relative downstream velocity is randomised, for shock velocity U in the rest frame, position R_9 , away from the compact object

$$\text{Then} \quad E = \frac{8.7 \times 10^8 \left[\frac{M}{M_\odot} \right]}{R_9} \text{ (eV)} \quad (1)$$

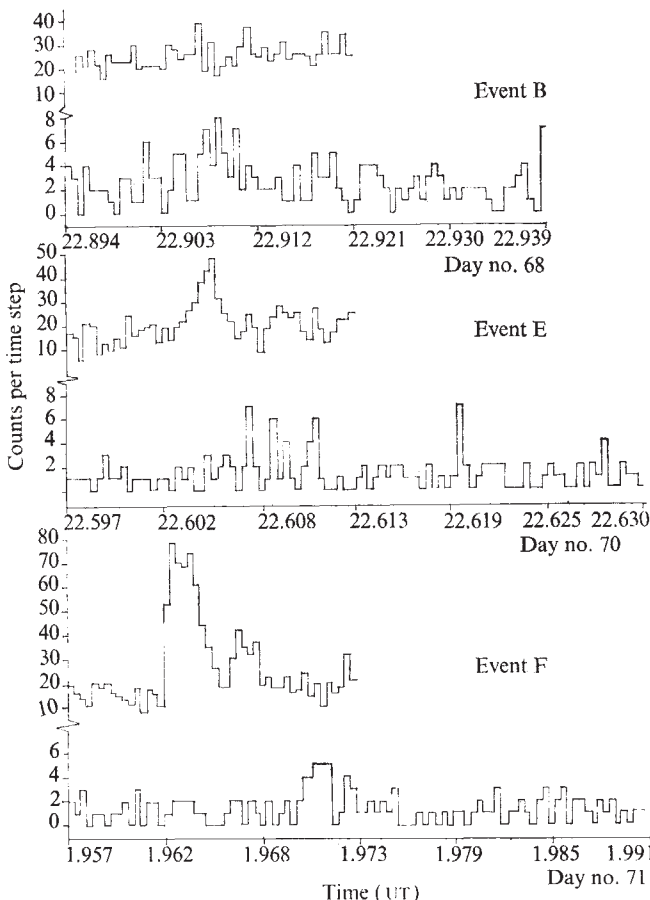
and

$$t = 1.3 (R_{9,0}^{3/2} - R_9^{3/2}) \left[\frac{M}{M_\odot} \right]^{-1/2} \text{ (s)} \quad (2)$$

is the time travel between $R_{9,0}$ and R_9 . If the object is a neutron star with magnetopause $\sim 0.2 R_9$, it is possible by free fall to get temperatures ~ 50 keV outside the magnetosphere. Equation (2) yields, however, a minimum delay between attaining 7 keV and 50 keV of $1.7(M/M_\odot)$ s, while the delay between 2 keV and 7 keV is $9.9(M/M_\odot)$ s. Thus it is difficult to explain observations where the low and high energy pulses are of comparable width unless a dramatic slowing of the shock by accreting material can be invoked. Increased energy in the higher channel does follow naturally from this mechanism, however.

Another possibility is to suppose that penetration of the magnetosphere by a large mass of transferring material occurs by some convection process (see ref. 4). Then this is followed by gravitational fall on to the neutron star atmosphere where the shock occurs to thermalise the energy. One must further suppose that deeper penetration at later times connects the material to different atmospheric conditions where the energy gets shared among fewer particles and hence a higher temperature results. Any short term periodicity (tens of seconds) can perhaps be related to the magnetosphere spin while long term periodicities

Fig. 1 Time profiles of three events showing scintillation telescope (ST) (top) data (50-190 keV) and collimated proportional counter (CPC) (bottom) data (2-7 keV) plotted on same timescale.



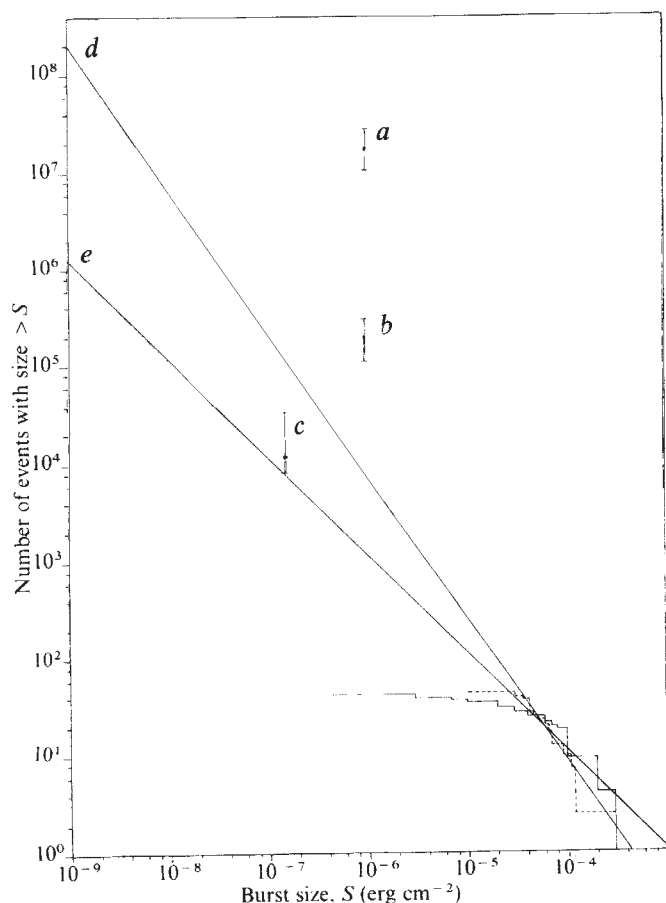


Fig. 2 Burst occurrence rates derived from our experimental results, for two source distributions; *a*, isotropic and *b*, galactic. Also illustrated for reference are the Vela (—) and Imp-7 (---) size spectra. *c*, From Imperial College balloon data. The two straight lines are extrapolations of the Vela/Imp-7 data assuming either *d*, $I^{-3/2}$ law or *e*, I^{-1} law. All rates have been normalised to Vela observation period July 3, 1969–December 23, 1973.

($\sim$ hours) could depend on the orbital period of the companion $\sim 4 \times 10^{10}$ cm away.

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Positions and evidence for a steady component for X-ray burst sources

DURING observations of the galactic centre region with the Ariel V RMC experiment we have observed X-ray flux from two sources within the error boxes for the burst sources MXB 1730–335 and MXB 1728–34. The observations were made in RMC mode and represent integrations over periods of about

one satellite orbit (100 min). This is distinct from the time mode used for the observations described in a companion paper (to be published) in which the time resolution is 32 s but with which no spatial resolution is possible.

The two sources were each observed at various times during the period 1976, 28 February to 8 March. The positions of these sources are

- (1) $\alpha_{1950} = 17 \text{ h } 30 \text{ min } 14 \text{ s}$ $\delta_{1950} = -33^\circ 21'$
- (2) $\alpha_{1950} = 17 \text{ h } 28 \text{ min } 29 \text{ s}$ $\delta_{1950} = -33^\circ 48'$

each with an error circle radius of $2'$. Position (1) lies wholly within the original error box¹ for the burst source MXB 1730–335 and overlaps the improved error circle given by Hearn² (Fig. 1). MXB 1730–335 is known to produce a rapid succession of bursts with an integrated intensity³ of 1.7×10^{-9} erg cm⁻² s⁻¹ (1.5–20 keV). Lewin *et al.* place an upper limit of twice this level on any non-burst activity from the source³. The intensity of the source which we observe corresponds to an energy of $\sim 3 \times 10^{-9}$ erg cm⁻² s⁻¹ over the range 1.5–20 keV, although this represents an extrapolation from the 2.9–7.6 keV range in which our observations were made. Thus the observed intensity is consistent with the reported burst activity, probably together with a steady non-burst component of about one half of the level of the upper limit found by Lewin *et al.* Thus the position quoted above almost certainly represents an improved determination of the position of MXB 1730–335. We note that the star cluster observed by Liller⁴ lies close to the centre of our error circle.

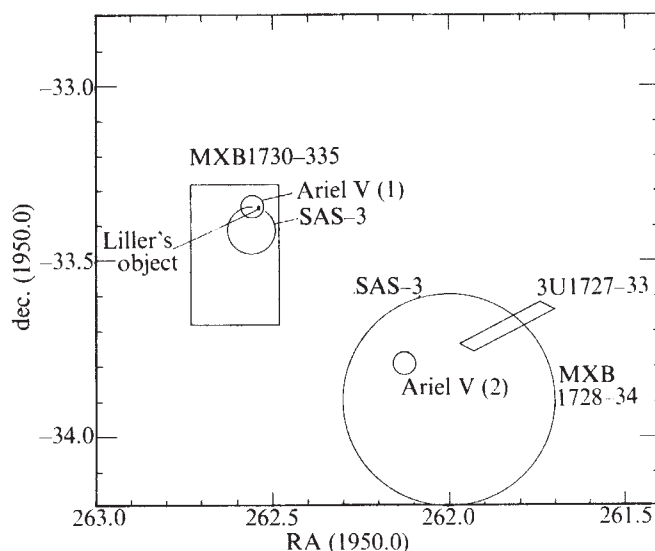


Fig. 1 90 per cent confidence error circles for the two sources reported here shown relative to the SAS-3 burst sources. The error circles should be shown as ellipses extended by 15% in the E–W direction.

The position of source (2) lies within the $18'$ radius error circle of MXB 1728–34 (unpublished). Only four bursts have been reported from this source, with a probable time between bursts of ~ 5 h. Each burst lasted only for a few seconds and the mean integrated flux associated with the bursts is at least two orders of magnitude lower than in MXB 1730–335. The intensity of source (2) integrated over one orbit was typically similar to that of source (1) although it was rather more variable. Thus there is no question of the observed flux being the integral of bursts of the type which have been reported for this source. If the source (2) represents flux from MXB 1728–34 then it must be due to a non-burst background emission.

We note that the position of source (2) lies close to the error box of the Uhuru source 3U1727–33. There is evidence for a source at position (2) in data collected in February–March, 1975, but we have found no evidence for flux from within the error box of 3U1727–33. Thus it is likely that 3U1727–33 lies outside its error box at position (2).

If 3U1727–33 and MXB1728–34 and our source (2) prove to be the same object then it will provide a useful clue to the nature of burst sources as 3U1727–33 has been shown to exhibit a periodicity indicating a ‘slow rotator’ type of source⁵.

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Observations of X-ray bursts from the vicinity of 3U1727–33

ARIEL V experiment C observed the region of sky near 3U1727–33 between March 8 and 10, 1976 and again between March 24 and 25. The experiment¹ consists of a multiwire collimated proportional counter with a field of view of 3.5° FWHM. The experiment is, however, offset from the spin axis of the satellite by 1.75°, so the effective field of view per spin cycle (4–5 s) is a Gaussian-like curve of 5.0° FWHM centred on the spin axis. The field of view during the present observations included the positions of 3U1727–33 (ref. 2), MX1716–30 (ref. 3) and two recently discovered burst sources, MXB1730–335 and MXB1728–34 (ref. 4).

Table 1 Data accumulated by experiment C on the Ariel V satellite

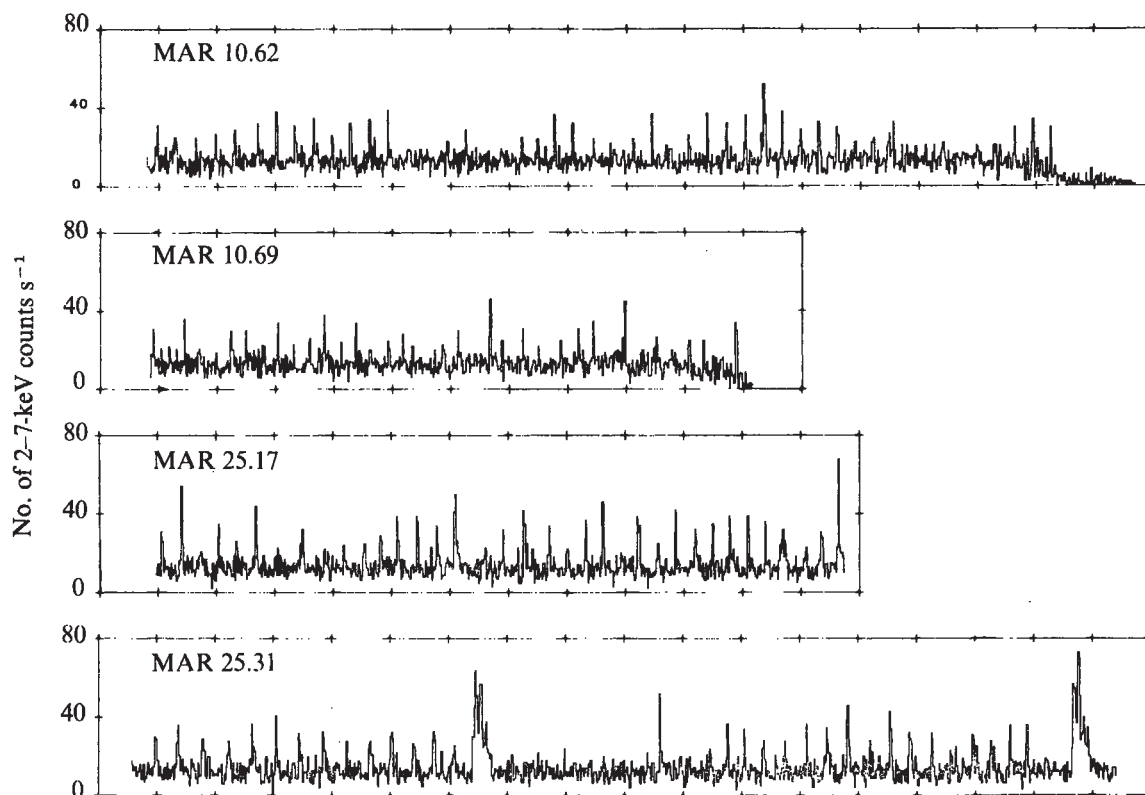
Time of burst (March 1976) (d)	Intensity ($\times 10^{-8}$ erg cm $^{-2}$)	Time resolution (s)	Notes
(1) 8.7764	20.0	32	
(2) 8.8910	5.2	32	Probably two pulses but maybe one.
8.8914	14.0	32	
(3) 9.1655	18.0	32	
(4) 10.3487	13.6	20	One long pulse or two close together.
(5) 10.6251	4.0	0.5	
(6) 10.9420	5.2	20	
(7) 11.0820	14.4	20	
(8) 24.7959	8.0	20	
(9) 24.8462	6.4	20	
(10) 24.8513	4.4	20	
(11) 24.8539	18.0	20	
(12) 24.9885	12.4	20	
(13) 25.2432	6.4	0.5	
(14) 25.2494	11.2	0.5	
(15) 25.3097	16.4	0.5	
(16) 25.3170	18.8	0.5	
(17) 25.3774	8.8	0.5	

Bursts listed above with 0.5-s time resolution are included because they are sufficiently strong to have exceeded the threshold set for detection with a 32 or 20 s accumulation interval. Numbers 15 and 16 can be seen in the lowest panel of Fig. 1.

Observations were made with a time resolution of 32 s, 20 s and 0.5 s in the 2–7 keV energy band. The mean counting rate from all the sources in the field of view, plus background, was ~ 12 counts s $^{-1}$ (the detector records ~ 100 counts s $^{-1}$ from the Crab Nebula in the same energy band).

The X-ray bursts seen by the ANS (ref. 5) and SAS-3 (refs. 4, 6) satellites generally last for of the order of a few

Fig. 1 Four orbits of experiment C data accumulated in 1-s intervals. The time of the beginning of each data record is given in days from the beginning of March 1976. The abscissae are graduated in 1-min intervals. The flux is plotted as experiment C 2–7 keV counts s $^{-1}$ with no aspect correction. On the same scale the Crab Nebula would register 100 counts s $^{-1}$ when aligned with the satellite spin axis. The field of view is occulted by the Earth towards the end of the records marked MAR 10.62 and MAR 10.69.



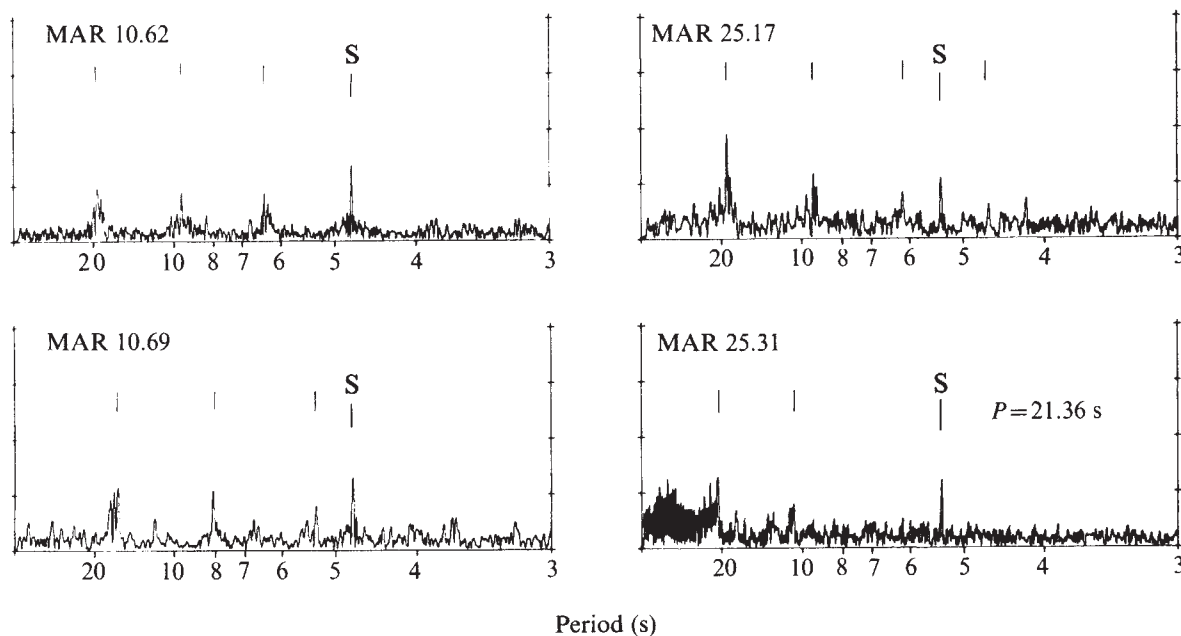


Fig. 2 Power spectra of the data shown in Fig. 1. Peaks near 20 s, plus their harmonics, are marked with vertical bars. The peak caused by the spin modulation is marked by a vertical bar with the symbol 'S' above.

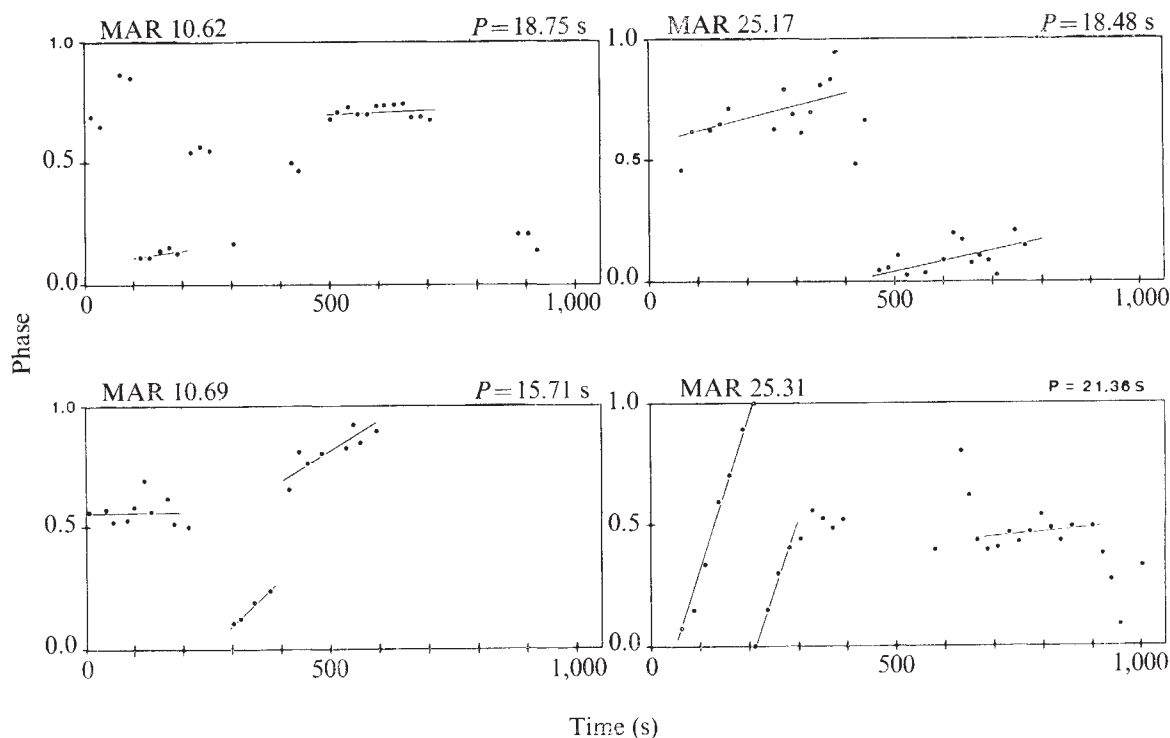


Fig. 3 The phase of bursts with respect to the folding period, P , is plotted against their arrival times (see text). Well defined burst trains are marked with straight lines.

seconds only, shorter than the 20 and 32 s accumulation intervals. The Ariel V data taken at these time resolutions can therefore only distinguish relatively intense or long lasting bursts. A threshold of 8 counts s^{-1} above the mean count rate was set as a criterion for identifying accumulation intervals containing such bursts. During a total of 17 h actual observing time, 17 of these events were recorded. The strength of each burst and the start time of the accumulation interval in which it was seen are listed in Table 1. Carpenter *et al.*⁷ have found a series of bursts originating from within the 17° FWHM field of view of experiment A which recur every 1.39 h. Using their epoch and period (G. K. Skinner, personal communication) we have computed the expected arrival times of bursts in the sequence for the experiment C observing intervals. None of the

events listed in Table 1 coincides with the times predicted by the sequence, whereas we should have observed 11 of these. The nearest, event 12 in Table 1, occurred 5 min later than the time predicted by the experiment A data, well outside the jitter observed by Carpenter *et al.*⁷. We note, however, that the first five events recorded by experiment C, which span an interval of 1.9 d, can be fitted by a period of 1.35 h with a jitter of $\pm 5\%$. One other event in the sequence should have been seen during the above interval and was not. Experiment A cannot collect data when experiment C is in a high time resolution mode, because of limits on spacecraft data storage, so two interpretations of the observations are possible: either the periodic bursting source seen by Carpenter *et al.*⁷ changed its mode of behaviour during the experiment C observing

periods or, more likely, the source lies outside the field of view of experiment C.

Examples of the data taken by experiment C at a time resolution of 0.5 s are shown in Fig. 1, accumulated in 1-s bins. The data show many significant bursts (> 3 s.d.) superimposed on the d.c. level of ~ 12 counts s^{-1} . Most of the bursts last only a few seconds, but some persist for up to ~ 20 s. The longer lasting bursts show clear evidence for spin modulation (period 4–5 s) indicating that they originate from a source which is offset from the spin axis of the satellite (RA 17 h 28 min, dec. -33.5°). The depth ($\sim 25\%$ peak to mean) and phase of the modulation are consistent with the position of MXB1730–335 (refs 3,7). On the other hand, the d.c. count rate shows no evidence of spin modulation and must originate primarily from a source close to the spin axis, probably 3U1727–33. The level of burst activity clearly diminishes following the longest bursts (for example the data beginning on March 25.31). This behaviour is reminiscent of the correlation between the size of bursts and their separation reported by Lewin⁴. It also suggests that both large and small bursts originate from the same source.

The spacing between many of the bursts in Fig. 1 is similar. This is confirmed by the power spectrum of each of the four data sets. These are shown in Fig. 2. In each case there is a well defined peak at a frequency corresponding to a period ~ 15 –20 s, with further peaks at the harmonics of this frequency. Note, however, that the primary peaks come at a different frequency in each of the four data sets. A peak is also seen at the spin period of the satellite and is marked by the symbol 'S'. This corresponds to the spin modulation noted earlier.

To further investigate this behaviour we have folded the arrival times of the bursts on a period close to that given by the power spectra in Fig. 2. We then plot the phase of the burst against its arrival time as shown in Fig. 3. On these diagrams, bursts which recur at the folding period lie on a horizontal line. Bursts which recur at a period slightly different from the folding period will lie on diagonal lines. It is evident that the bursts observed come in groups within which the separation of pulses remains approximately constant, the typical r.m.s. jitter being $\sim 5\%$. Transitions from one group to another, at a different phase and/or period, occur on a time scale short compared with the length of pulse trains.

In summary, therefore, we have observed, probably from MXB1730–335, sequences of periodic bursts which maintain their phase and period for several cycles and then may change abruptly in either phase or period. Longer lasting bursts (of the order of the separation of small bursts) are followed by relatively quiescent intervals. The abrupt changes in phase and period suggest that the burst trains are not produced by a rigid dynamical system such as a rotating star or orbiting stars; rather that they represent a characteristic oscillation period in the X-ray production mechanism or orbital motion in some non-rigid system such as an accretion disk. With regard to the latter possibility, and in view of the suggested identification of MXB1730–335 with a globular cluster^{8,9} we note that a time scale of ~ 20 s is the inner orbital period of a $\sim 10^6 M_\odot$ black hole ($t \approx 0.1 M_{BH}/10^6 M_\odot$ (s)).

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The radio tail galaxies in Abell 1314

THE study of radio tail objects is important to our understanding of extragalactic radio sources because the tail morphology allows inference of the temporal evolution of ejected radio components. Furthermore, with increased understanding of these objects, it may be possible to use radio tails as probes of extragalactic space in clusters of galaxies. All published models of radio tails require some form of streamlining resulting from the relative motion of the parent galaxy and the medium producing the tail shape. Therefore, to constrain the relevant dynamical quantities, we have obtained radial velocity measurements for 18 galaxies in the vicinity of A1314, including the two radio tail objects, IC708 and IC711.

The data were obtained with the Cassegrain spectrograph and RCA 33063 image tube camera mounted on the Steward Observatory 90" telescope. The dispersion used was ~ 240 Å mm^{-1} , which allowed redshift measurements using the H and K lines of calcium, as well as, in some cases, the 3,727 Å and 5,007 Å lines in emission. The results of these measurements, using the one-dimensional Grant Measuring Engine at the Kitt Peak National Observatory, are listed in Table 1.

Assuming all galaxies observed are cluster members except number 3 and number 19, we derive a mean cluster velocity of 10,160 $km s^{-1}$ and, assuming $H_0 = 50 km s^{-1} Mpc^{-1}$, a distance of 200 Mpc.

The radial velocity dispersion of the cluster, σ , is 708 $km s^{-1}$ and the total dispersion, σ_T , is 1,226 $km s^{-1}$.

Both radio tail galaxies, IC708 and IC711, have radial velocities fairly near the cluster mean

$$\langle v \rangle - v_r)_{IC708} \approx 1/3 \sigma$$

$$\langle v \rangle - v_r)_{IC711} \approx 1/2 \sigma$$

and, considering their proximity to the cluster centre, we therefore conclude that both galaxies are cluster members.

Confirmation of the cluster membership of IC711 strongly implies that *in situ* acceleration must be important in its radio components, as suggested by Vallee and Wilson¹. A model predicting such acceleration was suggested by Pacholczyk and Scott². Using the radial velocity data we may place strict constraints on the amount of acceleration necessary in IC711 and IC708, and, therefore, test the available radio tail models.

Table 1 Spectrographic measurements (see text)

Galaxy	α (1950) (h min s)			δ (1950) (° ' ")			Heliocentric velocity ($km s^{-1}$)
1	11	29	26	+49	17	10	9,363
2	11	29	51	+49	23	08	10,097
3	11	30	46	+49	30	54	318
4 IC708	11	31	16	+49	20	17	9,613
5 IC709	11	31	31	+49	19	09	9,549
6 IC711	11	32	03	+49	13	57	9,724
7 IC712	11	32	06	+49	21	14	10,054
8	11	33	01	+50	00	52	9,727
9	11	33	22	+49	43	38	9,467
10	11	33	49	+49	24	29	11,223
11	11	33	54	+49	20	23	9,641
12	11	32	59	+49	04	49	11,179
13	11	33	04	+49	25	39	9,870
14	11	32	47	+49	23	00	11,307
15	11	33	01	+49	26	34	10,454
16	11	29	26	+49	16	13	9,863
17	11	30	06	+49	22	34	11,251
18	11	30	34	+49	33	38	3,180

Assuming that the space velocity of IC711 relative to the cluster medium is σ_r , the angle between the velocity vector of this galaxy and our line of sight is 70° . In this case the radio tail is ~ 900 kpc long. Using these values for the galaxy velocity and tail length with Vallee and Wilson's estimate of the magnetic field ($H = 4 \times 10^{-6}$ gauss) we may estimate the importance of *in situ* acceleration for this radio tail.

From the equations for energy loss due to inverse Compton and synchrotron emission³ we calculate the e-folding decay time, t , for the energy of particles in the tail. Dividing the tail's length by the estimated velocity of the galaxies with respect to the centre of the cluster gives T , the length of time that particles emit detectable energy⁴. Then the ratio $N = T/t$ is a measure of the effect of *in situ* particle acceleration in the radio tail. For IC711 we find $N \approx 20$, a typical value from the Pacholczyk and Scott² theory. For IC708 a similar calculation yields $N \approx 4$.

From our spectroscopic study of galaxies in Abell 1314 we conclude that both reported radio tail galaxies are cluster members, and that *in situ* acceleration (with a magnitude consistent with Pacholczyk and Scott's² model) is an important effect in their radio components.

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White dwarfs, the Galaxy and Dirac's cosmology

AN apparent absence, or deficiency, of white dwarfs fainter than $\sim 10^{-4} L_\odot$ has been noticed for a long time¹. Often attributed to incomplete statistics for the coolest, faintest objects^{2,3}, this deficiency has nevertheless not been removed even after the most persistent spectroscopic surveys by Greenstein^{4,5} (see also Liebert⁶). Moreover, Jones's⁷ analysis of reduced proper motions for the very faint proper-motion stars observed by Eggen and by Luyten tends only to confirm the deficiency. If real, the lack of very faint white dwarfs may have its origin in a rapid drop of the internal heat content of the white dwarf as a result of ion crystallisation and of envelope convection when the star becomes sufficiently cool (see Greenstein⁴ and Van Horn⁸ and references therein). But perhaps it will eventually prove to be difficult, as Lamb and Van Horn⁹ have already found, to increase the cooling rate by a sufficiently large amount to account for all the deficiency. Another explanation is proposed here on the basis of Dirac's¹⁰⁻¹² cosmological hypothesis that the gravitational constant, G , varies with the elapsed time since the beginning of the expansion of the Universe as t^{-1} and the number of particles in the Universe increases as t^2 (if the measurements are made in atomic units). For a white dwarf, the Chandrasekhar mass limit is a collection of fundamental constants proportional to $G^{-3/2}$, and therefore increases with time as $t^{3/2}$. In the 'additive' version of Dirac's theory, the actual mass, M , of a relatively small object like a star remains essentially unchanged by the creation of new matter in the Universe, and so a white dwarf becomes 'more stable' as time goes on. But, in the 'multiplicative' version of his theory, M increases as t^2 and may eventually exceed the Chandrasekhar limit. If so, gravitational collapse of

the white dwarf into an invisible black hole or neutron star will quickly ensue. Since some possible empirical support has already been adduced for the multiplicative theory, although not for the additive theory¹³⁻¹⁷, it is interesting to determine whether the multiplicative theory may also have something to do with the apparent deficiency of faint white dwarfs and whether there are any possible consequences for galactic evolution.

First it is necessary to evaluate what would be the luminosity of a white dwarf (assumed to be in hydrostatic equilibrium) if the formal change of internal energy and gravitational potential energy of the star arising from the creation of new matter is available as free energy partly to raise the heat content of the star and partly to be radiated away at the surface, and is the sole source of visible luminosity. The changes of G and M are assumed to be given by

$$G(t) = G_0[t/t_0]^{-1} \quad (1)$$

and

$$M(t) = M_0[t/t_0]^2 \quad (2)$$

where the subscript 0 refers to the present epoch. Although these expressions give an undesirable zero mass and infinite G at $t = 0$, they are only needed at comparatively large fractions of t_0 , at which times they may serve as suitable approximations for the present purpose (Van Flandern's¹⁶ observed value for the residual secular acceleration of the Moon, interpreted as being carried by a decrease of G , is consistent with equation (1) for $t_0 = 12 \times 10^9$ yr). The mass-radius relationship¹⁸ relevant to most of the appropriate range of white-dwarf masses is approximately

$$\left[\frac{G}{G_0} \right]^2 \frac{R}{R_0} \approx \left[\frac{M}{M_0} \right]^{-1} \quad (3)$$

The luminosity follows from evaluating the rate of change of total (internal plus potential) energy

$$L = \frac{d}{dt} \left[\beta \frac{GM^2}{R} \right] \quad (4)$$

where β extends from $3/7$ to 0 , for masses ranging from very low values to values close to the Chandrasekhar limit, respectively; β may, however, be held constant here. The result is, at the present epoch

$$L = 3\beta GM^2/Rt_0 \quad (5)$$

For a typical white dwarf with $M = 0.8 M_\odot$, $R = 10^{-2} R_\odot$, and $\beta = 0.4$, and for an approximate age for the Universe of $t_0 = 12 \times 10^9$ yr (refs 16, 19), the expected luminosity is $L = 0.2 L_\odot$. (An analogous derivation for main-sequence stars shows that the 'extra' luminosity is insignificant, except for the faintest main-sequence stars; in general, it will be negligible for any star whose Helmholtz-Kelvin time, GM^2/RL , is short compared with t_0 .)

Since so high a white-dwarf luminosity is in serious conflict with observations², it may be concluded either that Dirac's theory is untenable, or that the radiation (and 'heating') is of an unknown type, or that the process of creation of new matter requires a corresponding supply of energy. For lack of a better alternative, I shall simply assume that the 'extra' energy somehow goes into work done creating the new matter and that the ordinary rates of stellar energy generation should be used to determine stellar temperatures and luminosities. Therefore, at least in its macroscopic effect, the process of creation is assumed to be a collective phenomenon involving the whole mass of the star; but this may be an unavoidable requirement of the multiplicative theory anyway, since Dirac¹² has already pointed

out that, in the case of the Earth, new atoms may have to form at the surfaces, rather than in the interiors, of rock crystals. In the case of gaseous stars, I shall follow Dirac in assuming that new atoms emerge with the same basic properties, that is, species, state, and general location, as the existing atoms.

To proceed further, simple physical assumptions about the structure and luminosity source of white dwarfs will be adopted here: (1) Chandrasekhar's¹⁸ classic mass limit of $1.44M_{\odot}$ for a white dwarf at the present epoch; (2) Mestel's²⁰ simplified cooling theory of white dwarfs, as presented by Schwarzschild²¹; and (3) an average mass of $0.8M_{\odot}$ for young (recently formed) white dwarfs. How this average mass has varied in the past is not easy to determine, but a good assumption is that it has been proportional to $G^{-3/2}$, since most critical stellar masses in astrophysics, such as the approximate upper and lower mass limits of ordinary stars^{22, 23}, have this proportionality.

Schwarzschild's²¹ standard relationship between the luminosity, L , of a white dwarf and its isothermal core temperature, T , is

$$L = KGMT^{3.5} \quad (6)$$

where K is a constant depending on the chemical composition of the radiative envelope. The luminosity of the white dwarf is supposed to be supplied solely by thermal cooling of the non-degenerate ions; the basic equation of thermal equilibrium is given by

$$\frac{dL(r)}{dM(r)} = -\frac{d}{dt}\left[\frac{3}{2}\mathcal{R}T\right] \quad (7)$$

whose correct integrated form is

$$L = -\frac{d}{dt}\left[\frac{3}{2}\mathcal{R}T\right]M \quad (8)$$

Solution of equations (1), (2), (6) and (8) for the core temperature can be simplified by assuming that the initial temperature is 'infinitely' hotter than the present temperature. Then the time, t_f , at which the white dwarf actually formed is found to be given by

$$t_f/t_0 \approx \exp(-t_{cl}/t_0) \quad (9)$$

where

$$t_{cl} = \left[\frac{3}{2}\mathcal{R}T\right]/(2.5L) \quad (10)$$

and all quantities in parentheses are evaluated at the present epoch. Note that t_{cl} is simply the classical cooling time that would apply if G and M remained constant in time. For the brighter luminosities characteristic of most of the observed white dwarfs, the revised cooling times are not much different from the classical cooling times and therefore do not destroy the approximate agreement with observations already achieved².

The most recent period at which a white dwarf with mass typical of its epoch could have formed to reach the Chandrasekhar mass limit at the present epoch follows from $0.8(t_f/t_0)^{3/2} = 1.44(t_f/t_0)^2$ and is $t_f = 0.31t_0$. Substitution of this result into equation (9) yields $t_{cl} \approx 1.2t_0$, which may be directly entered into Schwarzschild's²¹ Table 27.1 for the classical cooling times of white dwarfs as a function of their luminosities, if his cooling times are multiplied by a factor 1.3 to correspond to a mass of $1.44M_{\odot}$ and by another factor 0.3 to convert from a predominantly helium core composition to a more probable carbon core composition. With $t_0 = 12 \times 10^9$ yr, a value of $L = 2 \times 10^{-5}L_{\odot}$ is found for the present luminosity of an 'average' white dwarf that has cooled for $t_0 - t_f = 8 \times 10^9$ yr. Older white dwarfs would long ago have faded into invisibility through gravitational collapse. (Some older white dwarfs of smaller-than-average initial mass would still be visible, of course.) Most younger white dwarfs would

not yet have collapsed. It is interesting to note that most of the old white dwarfs with high space velocities^{1, 24} have large photometrically implied masses. Could some of these objects be on the verge of gravitational collapse? Accurate measurements of the surface gravities of the oldest white dwarfs will be necessary to determine whether these stars are, on the average, heavier than the brighter objects. Noticeable differences are expected to occur only for $L < 10^{-3}L_{\odot}$.

Allowance for a probable initial spectrum of white-dwarf masses, for the uncertainty of the adopted chemical composition, for known corrections to the classical white-dwarf mass limit, for various inadequacies of the white-dwarf cooling theory used here, and for the uncertainty of the adopted age of the Universe leads only to a general order of magnitude of 10^{-5} to $10^{-4}L_{\odot}$ as the predicted cutoff luminosity for most white dwarfs. This prediction seems to be in as good agreement with the available observations as could be expected.

The earliest generation of stars in the Galaxy probably formed within one galactic rotation period after the onset of collapse of the protogalaxy²⁵. At the present time, this period in the outer galactic regions near the Sun is 2×10^8 yr, but, in Dirac's theory, it must be scaled down as t , since the rotational velocity stays at the same fraction of the speed of light while the radius of the Galaxy changes as t . The first white dwarfs could have originated about one main-sequence lifetime (which also scales as t —see below) after that era. Since the collapse of the protogalaxy could have begun as late as $0.25t_0$ (ref. 19) the foregoing stellar formation time scales are seen to be relatively short in comparison, and the first-generation white dwarfs can therefore be expected to be, at the present time, invisible black holes or neutron stars with typical masses of $>1.6M_{\odot}$. Consequently, simple counts of observed stars in the solar neighbourhood will, if uncorrected for cosmological effects, lead to errors in the extrapolated numbers and masses of dead stars formed since the collapse of the protogalaxy. (The present discussion will ignore the probably small contribution to the mass of the Galaxy from black holes and neutron stars that have formed as direct end-products of normal stellar evolution.)

At first sight, it may seem that a gross underestimate must have been made in the standard extrapolation for dead stars²¹. That this is not so can be seen upon the following reflection. Consider the average properties of typical bright main-sequence stars that give rise to white dwarfs. Their average main-sequence lifetime, t_{MS} , at any epoch t may be regarded as short compared to t (since it is known to be short at the present epoch and it will be shown that t_{MS}/t remains approximately constant in time). Then their birthrate follows simply as $dN_{MS}/dt = N_{MS}/t_{MS}$, where N_{MS} is the number of such stars present in the Galaxy at time t . The next task is to evaluate $t_{MS} = qEM/L$ by noting that q , the mass fraction of hydrogen eventually consumed in the star²⁶, and E , the energy released per gram of material, are independent of t , while the average mass, M , of these bright main-sequence stars at the time of their formation (like all critical stellar masses) probably varies as $G^{-3/2} \propto t^{3/2}$ and their luminosity, L , scales as G^4M^3 (electron-scattering opacity) or as G^7M^5 (Kramers opacity). In either case $L \propto t^{1/2}$. Certainly the evolutionary change in L/M can be neglected, both because $t_{MS} \ll t$ and because this change will in any case be small ($G \propto t^{-1}$, $M \propto t^2$, and hence $L/M \approx$ constant). It then follows that $t_{MS} \propto t$. Next, the crude assumption is made that the ratio of the total mass in the form of bright main-sequence stars to that of interstellar gas in the Galaxy remains approximately constant after the collapse of the protogalaxy (anticipating the final result that the permanent loss of gas now tied up in dead stars is small). It then follows that $N_{MS} \propto t^{1/2}$, since the mass of the average bright star when it forms varies with the epoch as $t^{3/2}$ whereas the total mass of interstellar gas in the Galaxy increases as t^2 . The birthrate of bright stars becomes $dN_{MS}/dt \propto t^{-1/2}$.

The deathrate of these stars may be assumed to be equal to their birthrate, so that the rate of addition of mass in the form of new white dwarfs to the Galaxy is $0.8M_{\odot}(t/t_0)^{3/2}$ times

$(dN_{\text{MS}}/dt)_0(t/t_0)^{-1/2}$. The rate of growth of the masses of dead stars that have already formed follows from equation (2) as $2M_{\text{dead}}(t)/t$. The sum of these two terms yields the total rate

$$\frac{dM_{\text{dead}}(t)}{dt} = \frac{2M_{\text{dead}}(t)}{t} + 0.8M_{\odot} \left[\frac{dN_{\text{MS}}}{dt} \right]_0 \frac{t}{t_0} \quad (11)$$

By setting $M_{\text{dead}}(t) = 0$ at $t = t_i$ (corresponding to the end of the collapse of the protogalaxy) and by noting that the quantity

$$M_{\text{dead}}^* = 0.8M_{\odot}(dN_{\text{MS}}/dt)_0 t_0 \quad (12)$$

is the result of the standard extrapolation for dead stars, the solution of equation (11) is

$$M_{\text{dead}}(t) = M_{\text{dead}}^* [t/t_0]^2 \ln(t/t_i) \quad (13)$$

At the present time, $M_{\text{dead}} = M_{\text{dead}}^* \ln(t_0/t_i)$. Dead stars that formed at any time earlier than $t_i = 0.31t_0$ will now, however, be invisible objects rather than white dwarfs. If the total mass of dead stars at time t_i is multiplied by $(t_0/t_i)^2$, it follows that the present total mass of collapsed objects alone is $M_{\text{collapsed}} = M_{\text{dead}} \ln(t_i/t_0)$. Further, the present total mass of white dwarfs alone is found to be $M_{\text{WD}} = M_{\text{dead}}^* \ln(t_0/t_i)$, which is not significantly different from M_{dead}^* !

With reasonable choices for the collapse epoch, say $t_i/t_0 = 0.25-0.01$, the sought-for result is finally obtained: $M_{\text{dead}}/M_{\text{dead}}^* = 2-5$ and $M_{\text{collapsed}}/M_{\text{WD}} = 0.2-3$. These are modest values. Therefore, in the framework of Dirac's multiplicative theory, there is no special mechanism to produce the vast numbers of black holes or other dead stars that are sometimes suggested to compose the 'missing matter' in the solar neighbourhood and in the galactic halo.

The predicted paucity of stars during the past history of the Galaxy arises from the smaller galactic mass then, and implies that the Milky Way must have been considerably dimmer than it is at present. Most of the luminosity may be assumed to have come then (as now) from the few brightest stars. Assuming that these stars form with masses in proportion to $G^{-3/2}$ leads, as above, to $L \propto t^{1/2}$ and $N_{\text{brightest}} \propto t^{1/2}$, so that the total luminosity of the Galaxy, $N_{\text{brightest}} L$, is found to rise as t (but its colour probably changes relatively little with time). Thus, at the end of the original collapse phase of the Galaxy, after the initial metals abundance had been produced and supermassive stars were no longer being formed, the Milky Way would have been only 0.01-0.25 times as bright as it is today *ceteris paribus*. It could have been even fainter if the upper end of the allowable range of stellar masses were underpopulated when the galactic mass was small (an effect observed today in external galaxies of small mass). But, if the mass of the Galaxy had not been varying along with G , the Milky Way would have been brighter in proportion to t^{-1} in the past than it is today. Observations of distant spiral galaxies may eventually be able to discriminate between these various evolutionary models.

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Extraction of metals from basalt by humic acids

IN many studies, the activity of humic acid in weathering and other geological processes has been considered negligible. Only simple, low molecular weight organic acids are usually considered effective in mineral degradation¹. Those acids, however, frequently constitute only a minor fraction of the soil organic matter, particularly in semi-arid and arid areas. In Israel, fulvic acid/humic acid ratios in the organic matter of major soil types range, with one exception, from 1.5 to 6 (ref. 2). Humic acids are also well represented in the organic matter of many sediments^{3,4}. The effect of these high molecular weight compounds on mineral decomposition and metal dissolution is therefore of great interest in the earth sciences. The results presented here suggest that humic acids also may have a significant role in rock solubilisation and weathering, because of their capacity to extract considerable amounts of metals, particularly copper and zinc, from basalt rock.

Though the part played by fulvic acids and other low molecular weight organic acids in weathering has been widely documented, the action of humic acids in these processes has been examined in only a few cases. Schalscha *et al.*⁵ extracted considerable amounts of Fe from minerals treated with humic acids. The solubilisation of major elements from aluminosilicates has been shown to be considerably speeded up by treatments with humic acids or organic acid components of humic acid⁶⁻⁸.

Baker⁹ has reported on the strong solvent activity of humic acid from a podzolic soil in Tasmania, towards a number of minerals and metals. In his studies, separate mineral species were exposed to the action of humic acids. Here the effect of humic acids on basalt rock has been examined with the purpose of evaluating their role in the supply of soluble metals to soils.

Figure 1 shows that after a rapid initial rise, the dissolution rate of nearly all elements decreases rapidly. The dissolution capacity of the three humic acids is fairly similar. Ca, Mg, Co and Ni came to near equilibrium after 6-12 h, Al and Zn after 36 h. Fe, Mn, Cr and, to a lesser extent, Cu continue to be dissolved, though at a slow rate, after 108 h. Fe was the most extracted metal, followed by Al, Ca and Mg (Table 1). The transition metals were extracted in trace amounts. Relative to their contents in the basalt, Cu was the most extracted metal, followed by Zn. Next came Co, Cr and Mn, then Ni. Less than 0.5% of Fe, Al, Mg and Ca were extracted by the humic acids. Arranged according to the relative amounts extracted, the following sequence is obtained: Cu > Zn > Mn > Cr > Co > Ni > Al > Fe > Mg > Ca. The large amounts of metals extracted by the humic acids indicate the significant role these organic compounds may have in weathering and soil formation. An even more important aspect of that capacity is the role humic acids may play making micronutrients available (particularly copper and zinc) to plants growing on basalt-derived soils.

The capacity of humic acids to dissolve metals has been given as up to 682 mg g⁻¹ (ref. 4). Thus, the total amounts

Table 1 Extraction of alkaline earths and metals from 1 g basalt after 108 h treatment with three humic acids

	Basalt (mg)	Humic acid 1		Humic acid 2		Humic acid 3	
		µg	%	µg	%	µg	%
Al	63.7	387	0.61	402	0.63	366	0.57
Fe	110.1	550	0.50	491	0.44	499	0.45
Ca	71.4	297	0.42	265	0.37	281	0.39
Mg	34.7	175	0.50	169	0.49	153	0.44
Co	41×10^{-3}	1.1	2.7	1.3	3.2	1.9	4.6
Cr	221×10^{-3}	7.9	3.6	7.6	3.4	7.2	3.3
Cu	59×10^{-3}	7.7	13.1	6.5	11.0	6.4	10.9
Mn	141×10^{-2}	48	3.4	53	3.8	47	3.3
Ni	211×10^{-3}	2.3	1.1	2.2	1.0	3.4	1.6
Zn	118×10^{-3}	5.5	4.7	6.8	5.8	6.7	5.7

dissolved in this experiment—10–15 mg g⁻¹ humic acid—are far below the dissolving capacity of the acid. Incongruent dissolution of components from various aluminosilicates by organic acids has been shown by Huang and Keller⁷. From most of the minerals, alkalis were removed preferentially so

that the mineral surfaces remained enriched in Si–Al relative to the inner core. Further dissolution proceeds probably by diffusion through the residual envelope and is consequently slowed down considerably, or stopped altogether, as is the case with Ca and Mg in the experiment reported here. Fe and Mn, and to a lesser extent some of the other transition metals, particularly Cr, remain in basalt partly in the free oxide minerals magnetite and ilmenite, from which they continue to be dissolved, though at a slow rate.

Incongruent dissolution is probably also related to the relative affinity of the cations for the ligands of the humic acid. Though the relative stabilities of metal–fulvic acid complexes have been studied extensively, only fragmentary data are available on metals–humic acids complexes. Courpron¹¹ has given stability constants of log *K* = 7.00 and log *K* = 2.87 for complexes of soil-derived humic acid with Cu and Zn, respectively. The following order of complex stability at pH 7 for soil humic acids and metals has been established by Verloo and Cottenie¹²: Zn > Cu > Pb > Fe > Mn. If the coagulation capacity of metals for humic acid is an indicator of their affinity for humic acid, the following series obtains⁴: Al > Fe > Cu > Co > Ni. The effectiveness of added metals in coagulating humic acids derived from soils follows the order¹⁰: Al > Fe > Cu > Zn > Ni > Co; and a similar sequence obtains in the order of magnitude of the pH drop of humic acids on addition of metallic cations. The relatively strong extraction of Cu and, to a lesser degree, of Zn from the basalt may therefore be explained by the strong affinity of those metals for humic acid.

Fe and Al were less extracted than may have been anticipated from their relative affinities for humic acid; it is very likely that the nature of the solid phase also influences the rate of dissolution. Complexes which may be theoretically possible on the basis of strictly chemical considerations may in fact not form because of geometric hindrance or bond strength⁶. Thus, in strongly complexing organic acids, the dissolution of Al progresses differently in labradorite and augite: in labradorite Al is retained, whereas in augite it is removed, though less readily than other cations⁷.

Differences in the degrees of decomposition of minerals by organic acids have been explained by Ginzburg *et al.*⁶ as consequent upon different relative stabilities. The relatively low extraction of iron is probably related to the relatively high Gibbs energies of formation of magnetite and ilmenite (two important host minerals of Fe in basalt) compared with the much lower formation energies of the aluminosilicates, olivine, pyroxene, in which most of the transition metals are contained.

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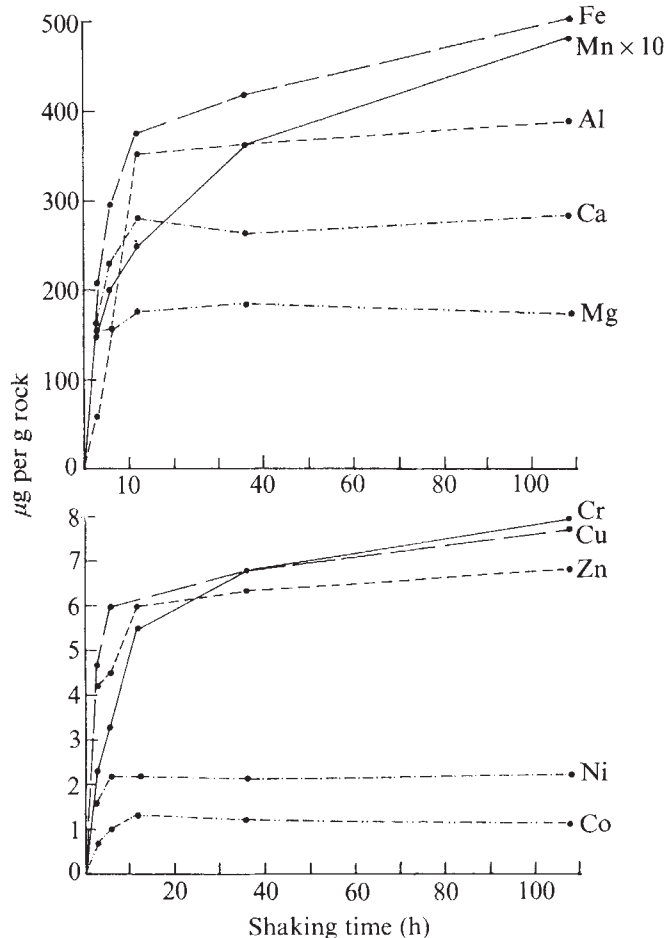
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Fig. 1 Extraction of alkaline earths and metals from basalt shaken for up to 108 h with humic acid. The humic acids were extracted from three Mediterranean soils: protogrumosol derived from basalt, under *Eucalyptus rostrata*; Mediterranean Brown Forest Soil derived from hard chalk, under annual grasses; and Rendzina, derived from soft chalk, under *Pinus halepensis*. Extraction, isolation and purification of the humic acids were similar to the methods described by Khan¹⁰. Alkaline olivine basalt, chemical composition given in Table 1, was pulverised in a Fritsch agate ball mill and then sieved through a 200 mesh Spex nylon sieve. The humic acid was redissolved in a NaCl solution to give a 0.1% w/v humic acid in 0.01 M NaCl final solution, and adjusted to pH 6.5. One gram of rock powder was shaken for 3, 6, 12, 36 and 108 h with the humic acid solutions at 25 °C, and the soluble elements were determined by atomic absorption. Controls of rock shaken with 0.01 M NaCl alone were run separately. Also determined was the metal content of the humic acids alone.



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Climatic information from $^{18}\text{O}/^{16}\text{O}$ ratios of cellulose in tree rings

THE high precision of dendrochronology makes possible the construction of short ($\sim 9,000$ yr), detailed records of past climates. This paper explores the possibility of using the oxygen isotopic composition of cellulose from tree rings as a 'thermometer' to measure past temperatures. Using meteorological data from Edmonton we have shown that temperatures can be measured with a precision of $\sim \pm 0.15^\circ\text{C}$ when averaged over a 5-yr period.

To investigate the variation of the ^{18}O -content of cellulose with temperature, we have studied a white spruce (*Picea glauca*) which grew in Edmonton, Alberta from 1882 to 1969. Detailed climate records for this location have been kept since 1880. Samples were taken from a 5-cm thick transverse section

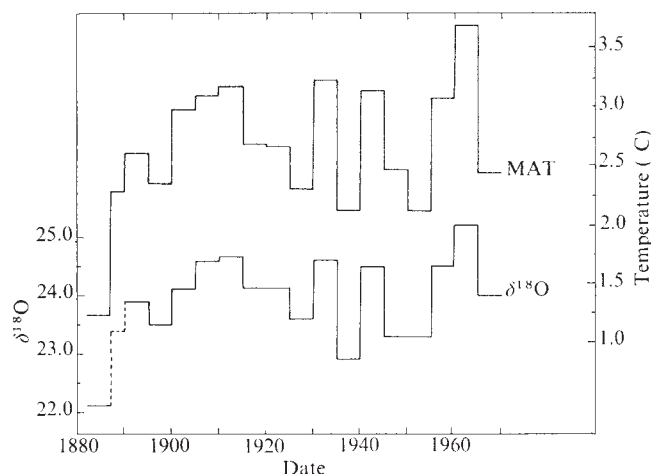


Fig. 2 A comparison between the mean annual temperature (MAT) curve obtained from the meteorological data and that obtained from $^{18}\text{O}/^{16}\text{O}$ ratios of cellulose. (The broken part of the isotope curve is calculated for some out-of-sequence rings that were not analysed.)

The $\delta^{18}\text{O}$ values range from 22.1 ‰ (1.23 °C) to 25.2 ‰ (3.64 °C). The temperature coefficient is 1.3 ± 0.1 ‰ per °C in reasonable agreement with the value of 2.2 ± 1.0 ‰ per °C obtained for algae³. The precision of the $^{18}\text{O}/^{16}\text{O}$ measurements corresponds to an uncertainty of $\pm 0.15^\circ\text{C}$ in mean annual temperature.

The choice of growing period over which the temperatures are averaged is somewhat arbitrary but the September to August and January to December 5-yr means differ on average by only 0.1 °C. Table 1 shows the correlation between $\delta^{18}\text{O}$ and mean temperatures calculated for different growth periods. It is apparent that the best correlation occurs with mean annual temperatures for September to August. The correlation with mean summer temperatures is poor.

There are two possible explanations for this. First, in addition to the temperature effect on the fractionation factor, there is also variation in the isotopic composition of the source water being used to produce cellulose. This is also climate dependent⁴. Ruben⁵ has shown that oxygen transpired during photosynthesis is derived only from water. It thus seems fair to assume that the cellulose oxygen is derived from CO_2 . It is possible, however, that isotope exchange can occur between CO_2 and H_2O before photosynthesis occurs so that the isotopic composition of the CO_2 will reflect that of the source water ($\alpha_{\text{CO}_2-\text{H}_2\text{O}} = 1.0407$ at 25 °C (ref. 6)). Thus it is possible that winter precipitation stored as soil water after melting may have a significant role in the production of cellulose during the growing season. The results shown here suggest that the contribution from variation in source water is relatively small, however, since the temperature dependence of the fractionation factor is approximately four times as large as the observed variation in the Edmonton area (0.3 ‰ per °C, calculated from data in the International Atomic Energy–World Meteorological

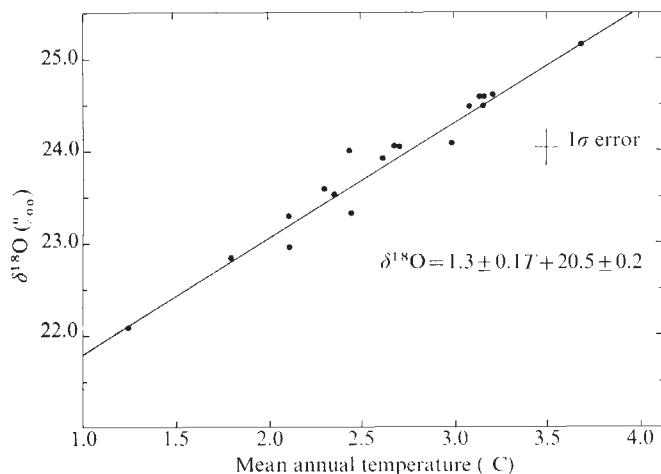


Fig. 1 The variation of $\delta^{18}\text{O}$ (SMOW) with September to August mean annual temperature for 5-yr periods. The equation of the line was obtained by a least-squares fit to the data points.

of the trunk cut ~ 1.5 m above ground. A 1-cm wide slice was cut from the section, divided into 5-yr groups of rings and the α -cellulose from each group extracted by standard chemical procedures¹. After washing and drying in a vacuum oven (50 °C for 5 d), the cellulose was burnt in an evacuated nickel reaction vessel at 1,100 °C for 60 min. The water–gas reaction occurs producing a mixture of CO , CO_2 and H_2 . The hydrogen produced diffuses through the walls of the reaction vessel forcing the reaction to completion. (This technique was developed at the California Institute of Technology by P.T. and S. Epstein). The CO was converted to CO_2 and C in a high voltage discharge produced between platinum electrodes. Yields of CO_2 were normally 98–100% of the theoretical predictions. All samples were run as consecutive replicates and no significant memory effects were observed.

The isotopic composition of the CO_2 was measured using a 12" radius, double collecting isotope ratio mass spectrometer. Results are expressed as a δ value ‰ with respect to standard mean ocean water (SMOW)² ($\delta^{18}\text{O}_x = [(^{18}\text{O}/^{16}\text{O})_x / (^{18}\text{O}/^{16}\text{O})_{\text{SMOW}} - 1] \times 1,000$ ‰). The precision of the replicate analyses was generally better than ± 0.20 ‰.

The results show a good correlation between the $^{18}\text{O}/^{16}\text{O}$ ratio and mean annual temperature calculated on the assumption that growth in any given year stops in September (Figs 1 and 2).

Table 1 Correlation between $\delta^{18}\text{O}$ and mean temperatures calculated for different growth periods

Temperature (5-yr mean)	Correlation coefficient $\delta^{18}\text{O}$ against temperature
Mean summer (May, June, July, August)	0.24
Mean winter (1) (January, February, March)	0.82
Mean winter (2) (December, January, February, March)	0.86
Mean annual (1) (January–December)	0.94
Mean annual (2) (September of previous year to August of final year)	0.97

Organization survey 1969, 1970, 1971 (ref. 7)). Second, it has been shown that net photosynthesis and growth in coniferous trees depends markedly on the production of stored foods (sugars from photosynthesis) during the winter⁸, so that winter temperatures may have a direct bearing on the isotopic composition of the cellulose produced the following year.

It is apparent that much work remains to be done to understand fully the isotopic processes involved in the production of cellulose. The empirical results presented here, however, show that the temperature dependence of the isotopic fractionation can be measured and that the climate record in the cellulose is not destroyed by subsequent exchange over a period of up to 90 yr. Measurements of D/H ratios of cellulose are under way in this laboratory to provide an independent cross check with the oxygen data.

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Geometry of river meanders

THERE are many factors affecting the geometry of rivers. Width, depth, slope, flow velocity and plan shape are all influenced by discharge, sediment load, sediment types, and valley slope through the operation of the continuity, flow resistance, bed load, bank competence and meander equations. If each of these equations could be determined accurately then their simultaneous solution would define the three-dimensional geometry of alluvial channels^{1,2}. Although the equations cannot be specified accurately the basic mechanical principles underlying most of the relevant processes are understood. The one exception is the process responsible for plan geometry. Indeed, many theories have been presented to explain the development and maintenance of these patterns, with varying degrees of success. I show here that meander arc length may be a unique function of channel width.

Yalin³ has proposed that the occurrence of meanders could be attributed to the law governing the spatial correlation among perturbations in turbulent flow. He demonstrated that the behaviour of the largest macroturbulent eddies would be systematically affected by a permanent discontinuity, and that this would result in the same and reverse kind of disturbance occurring alternately at equal length intervals along the direction of flow. On the basis of his theoretical analysis the wavelength of meanders, λ , in fully developed turbulent flow was related to the width of the channel, w , through

$$\lambda = 2\pi w$$

If turbulence were not fully developed, he has argued, the wavelength would be dependent on the Reynolds number and

the relative roughness of the section. For width/median bed material grain size values greater or equal to a thousand, he has argued that

$$\lambda = 20w$$

Leopold and Wolman's⁴ field data plot within these limits, which seems to substantiate Yalin's hypothesis. But as turbulent flow is normally fully developed at bankfull stage—the channel-forming discharge—the coefficient in Leopold and Wolman's best-fit relationship of

$$\lambda = 10.9w^{1.01} \text{ feet}$$

seems rather high.

This discrepancy between theory and practice could be explained by the fact that the repeating distance between discontinuities should be measured along the channel and not as a straight line distance. To check this hypothesis I have compared data from the rivers Wye and Tweed with Leopold and Wolman's data.

First, their equations linking wavelength with channel width and radius of curvature, r , were solved to obtain the relationship between radius of curvature and width

$$r = 2.37w^{1.03} \text{ feet}$$

As the exponent of this equation was not significantly different from unity, a linear relationship was assumed and the slope adjusted accordingly

$$r = 2.40w \text{ feet or m}$$

Most of the data for the Wye and Tweed plot above this line (Fig. 1) immediately indicating that Leopold and Wolman's curve is not a general one. This scatter was rationalised when information on meander arc angle, θ , measured between inflexion points was included. Leopold and Wolman's curve seems to relate to well developed meanders (150°) because points further above the line have progressively smaller arc angles. On the assumption that the arc angle for their curve is 150°, the relationship between arc length, z , measured between inflexion points, and channel width can be obtained

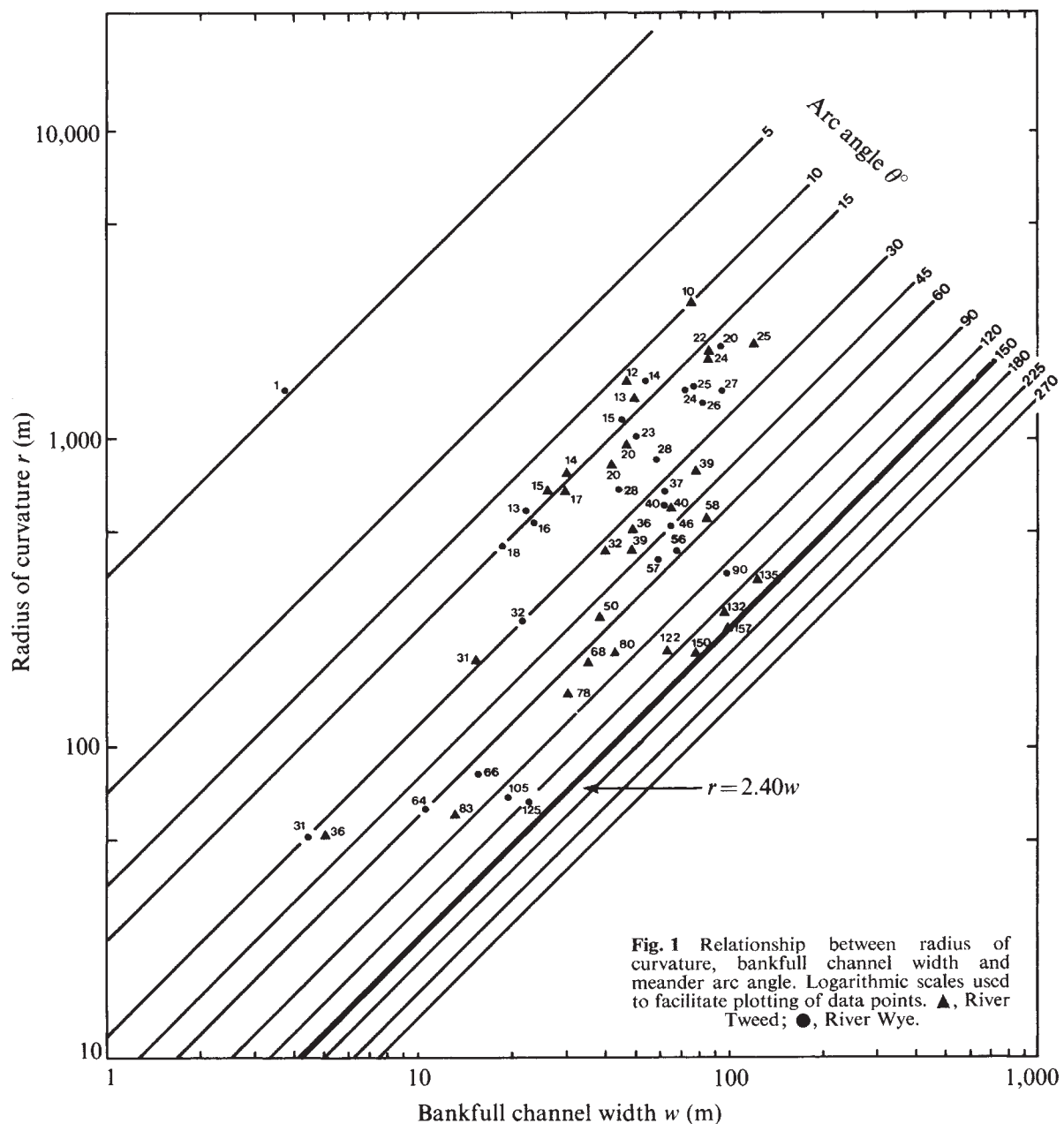
$$z = 6.28w \text{ m} \\ = 2\pi w$$

If this is a general relationship for all channels it would explain the plotting positions of the Wye and Tweed data on the graph of radius of curvature against channel width. Substituting z for w allows a family of curves to be constructed for different arc angles (Fig. 1). Field data corroborate this substitution,

Table 1 Relationships between wavelength, radius of curvature and bankfull channel width for various arc angles*

Arc angle (θ°)	Value of k in $r = kw$	Value of m in $\lambda = mr$	Value of n in $\lambda = nw$
270	1.33	2.83	3.77
225	1.60	3.70	5.91
180	2.00	4.00	8.00
150	2.40	3.86	9.27
135	2.67	3.70	9.85
120	3.00	3.46	10.39
90	4.00	2.83	11.31
60	6.00	2.00	12.00
45	8.00	1.53	12.25
30	12.00	1.04	12.42
15	24.00	0.52	12.53
10	36.00	0.35	12.55
5	72.00	0.17	12.56
1	360.00	0.04	12.57

* $k = 360/\theta$; $m = 4 \sin(\theta/2)$; $n = (1,440/\theta) \sin(\theta/2)$.



so the relationship between meander arc length and channel width seems to be general. Other research workers have noted that the spacing of riffles in straight and meandering channels is between five and seven channel widths⁵ and that skew shoals in flumes have been found to be 6.5 channel widths apart⁶.

This analysis indicates that the repeating distance measured down channel is $4\pi w$ which contrasts with Yalin's value for wavelength of $2\pi w$. Given that it would be more appropriate to use channel distance rather than plan distance in the calculation of repeating length, Yalin's model underestimates the repeating length by a factor of two.

How can this be explained? Closer inspection of Yalin's model indicates that he calculated repeating lengths by assuming that the largest macroturbulent eddy was equivalent to the width of the channel. Physically, this eddy must be interpreted as a helicoidal flow cell, and, as it systematically changes polarity, it influences the local pattern of accelerations and retardations. Approaching a pool (meander apex) the strength of the secondary circulation increases to a maximum with surface flows directed towards the eroding bank. Immediately downstream the polarity of the cell is reversed and the process repeated (Fig. 2a).

This pattern of secondary flows is probably incorrect, however. At high flow stages two cells, exhibiting surface flow

convergences, have been identified at the pool (meander apex); whereas at the riffle (inflexion point) two cells, with surface flow divergence, have been observed⁷ (Fig. 2b). On the assumption that the average size of this macroturbulent eddy is half the channel width, recalculation gives a repeating length of $4\pi w$.

As theory and practice indicate a general relationship between meander arc length and channel width it is possible to define the relationships between wavelength, radius of curvature and channel width for different arc angles (Table 1). This explains why empirical equations are specific to a given set of data unless meander arc angle is invariant.

Although this model helps to explain the regularity of channel patterns it does not successfully explain the initiation of meanders. A local increase in bank roughness is required to produce the asymmetry of flow but it does not explain how this can be achieved in homogeneous material. This approach merits further research however, particularly on the generation and effect of secondary flows on natural channels.

Even though it is not possible at present to explain meander mechanism, it is necessary to define the plan geometry of river channels for design purposes. Sinuosity, defined as the ratio of channel length to valley length or valley slope to channel slope, is probably the best measure because it is related to

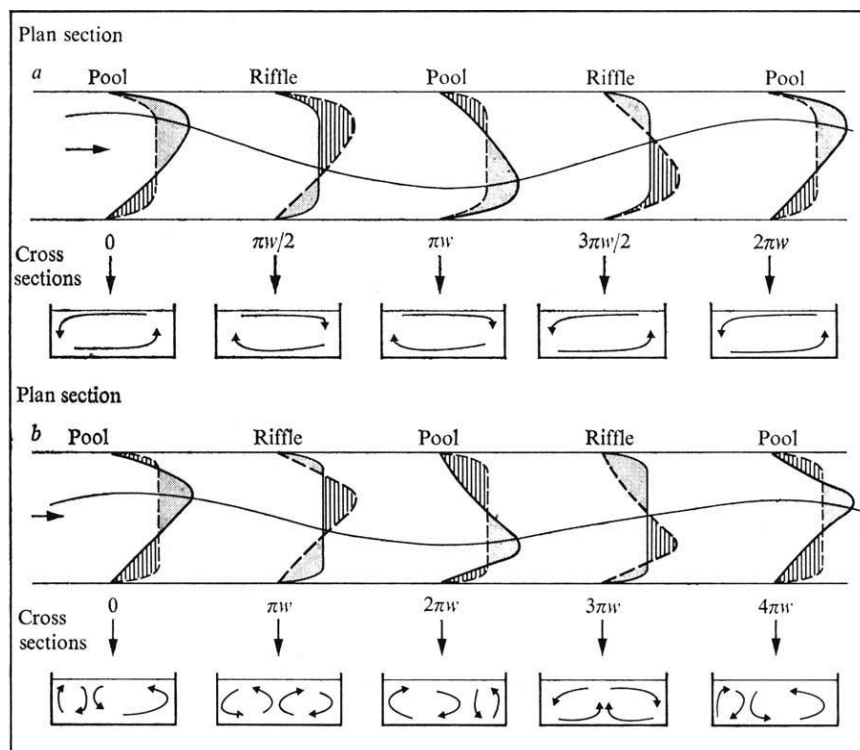


Fig. 2 Size of macroturbulent eddies in natural channels. *a*, Single secondary cell (after Yalin³); *b*, twin secondary cells (after Hey and Thorne⁷); —, surface velocity profile at section; --- surface velocity profile at neighbouring upstream section; stippling, accelerated surface flow; vertical lines, retarded surface flow.

meander processes. Any change in channel slope, through erosion or deposition, to a value defined by the bed load transport equation will result in a new plan form because valley slope can be considered invariant for channel design purposes. Unfortunately, for a given value of sinuosity an almost infinite number of patterns are possible (Fig. 3). If the

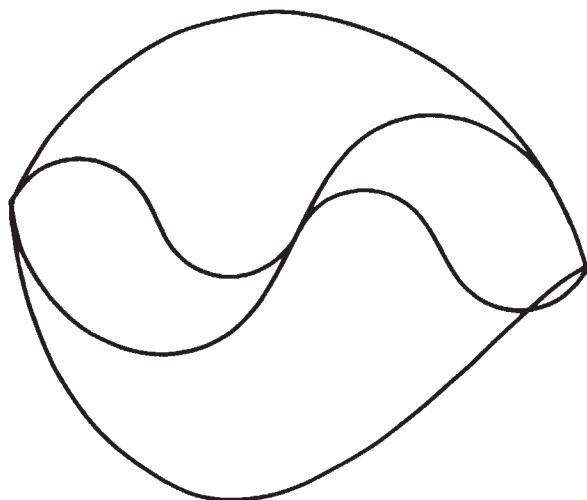


Fig. 3 Multivalued plan geometry for a given sinuosity (1.5 in case illustrated).

meander geometry is to be specified accurately then it will be necessary to determine the size of each meander arc. As arc length is a unique function of channel width, which in turn is defined by the bank competence equations, a determinate solution is possible.

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New hominid from Lake Ndutu, Tanzania

THIS report describes the stratigraphical and archaeological position of an hominid skull discovered in Pleistocene deposits around Lake Ndutu, on the Serengeti Plains (3°00'S; 35°00'E). The skull itself is described elsewhere¹. Lake Ndutu, together with the adjacent Lake Masek, is geomorphologically a continuation of the Olduvai Pleistocene deposits. The names of both lakes have been used to designate the upper parts of the Olduvai Sequence—the Ndutu and Masek Beds (formerly bed V and bed IVB, respectively); both are soda lakes.

Lake Ndutu is the smaller of the two (Lake Masek had enough water during the dry season of 1974–September–November—to support a resident population of hippopotami), and shows very marked fluctuations in water level between the wet and dry seasons. During the wet season the whole lake shore is under water, whereas during the dry season the water recedes mostly southwards and eastwards to expose the larger part of the western and northern shores of the lake. The exposed western flats are littered with lithic and faunal materials.

The site was first visited during September 1972, when a one-week preliminary survey was undertaken. This work included random surface collections in seven areas, each 2 m in radius, and the excavation of 2 m × 2 m trial trench with the objective of elucidating the stratigraphy of the site. During September and October 1973, the Tanzanian Department of Antiquities undertook the initial excavation of the site. These excavations, apart from exposing occupational floors with *in situ* lithic and faunal materials, also exposed a hominid skull¹.

The Ndutu deposits comprise a claystone member which can be subdivided into a greenish sandy clay unit, 15–45 cm thick, underlain by about 60 cm of sand-free, green clay with chert nodules in the lowest levels (R. Hay, California

Univ., Berkeley, private communication). The sandy clay unit can be further subdivided into sandy clay and silty clay subunits. To the north, along the shore, a hard, re-worked tuff, 10–12 cm thick, overlies the claystone. The occupational floor containing the skull rested on the silty clay subunit, and below it, resting on sand-free, green clay, were *in situ* lithic and faunal materials of what has been tentatively designated the second occupational floor.

The surface of the western margin flats are littered with lithic and faunal material but one problem concerns its provenance. Although the water level fluctuates at present it is not at all clear whether a similar situation obtained during the period when early man used either the lake or its vicinity as camping and hunting grounds. Did early man camp at the site seasonally during lacustrine recessions, or did he camp in the area when the lake was either non-existent or had dried up during such climatic oscillations as are known to have occurred elsewhere in the Pleistocene epoch? Or has the lithic and faunal material been brought into the area from elsewhere?

During the excavations it was found that there is a noticeable disconformity between the sand-free, green clay unit, and the overlying greenish, sandy-clay unit. Several small channels were cut into the green clay, in some cases to a depth of approximately 30 cm. Lithic and faunal materials were found at the bottom of the channels. It is, therefore, possible that early man camped or hunted at the lake shore when there was a minor climatic change which either resulted in the lake temporarily drying up or the level of the lake receding. This hypothesis is supported by the fact that it is only the sandy clay unit which is fossiliferous and contains implements. Although some of the artefacts are rolled it is nonetheless possible that both the lithic and faunal materials are archaeologically *in situ*; the rolling could be a consequence of the constant seasonal movements of lake currents.

During September and October 1973, an area of approximately 140 m² was excavated. The excavated deposits were situated in an area containing considerable amounts of surface lithic and faunal material. An area of approximately 80 m² included a high density of *in situ* lithic and faunal materials. It was in this area that the first and second occupational floors were exposed.

The *in situ* lithic materials are characterised by a very low percentage of finished tools. In the first floor, among the 270 plotted lithic and faunal materials only 20 are definitive tools (about 7.7%). Among the tools, spheroids and hammerstones predominate. There are six flakes in all, three of which are regular—two triangular and one rectangular. The remaining three are irregular. The cores are amorphous. Quartz and quartzite are the predominant raw material. The loose finds from within the sandy clay subunit overlying the first floor confirms this analysis. They include five cores, four regular flakes (one with retouched edges), three irregular flakes, one core scraper, two quartz pebbles probably used as hammerstones, and twenty-two waste flakes.

The tools represented in this collection are rather non-descript and of an indeterminate industry. Although hand axes and cleavers occur in the surface material, they are few, and even among the surface collections spheroids seem to be the predominant tool type, with cores also well represented. The absence of hand axes and cleavers among the *in situ* artefacts raises the question of the type of industry represented. The type of hominid represented by the skull is generally thought to be associated with the Acheulean industrial complex. The tentative date of the site as well as the mineralogical contents of the deposits also place the site within the chronological boundaries of the Acheulean industrial complex in the Olduvai Gorge and other sites in Tanzania and eastern Africa. Two possible hypotheses can be used to explain the absence of the tool types widely found

in the Acheulean industrial complex. Either the assemblage belongs to a different cultural and technological continuum and belongs to an industry post-dating, or contemporaneous with, later phases of the Acheulean industrial complex, or it belongs to the Acheulean, and the area excavated is only an indication of activity patterning. The latter hypothesis seems to be the most likely, and is supported by the fact that hand axes and cleavers, though few, are represented in the surface materials; moreover, excavation of a small trench on the site revealed an *in situ* hand axe (M. D. Leakey and R. Hay, private communication). The presence of a very high percentage of faunal materials in the collection—about 94 out of the 270 plotted *in situ* materials of the first floor are splinters and sizeable bone pieces including horns and antlers—seems to suggest that the area was probably a butchering site, though the presence of a hominid skull among the collection in such a context would be rather enigmatic.

The tuff overlying the claystone probably represents the Norkilili Member of the Masek Beds of Olduvai, though the possibility that it represents the lower unit of the Ndutu Beds cannot be ruled out (R. Hay, private communication). One preliminary chronometric date has been obtained from bone found in the first occupational floor, and preliminary racemisation measurements suggest a general age of ~500,000–600,000 yr (J. L. Bada, private communication). A sample of bone from the Masek Beds of Olduvai has given similar results.

I thank Mr F. T. Masao, the Curator of the National Museum, who participated in the 1972 preliminary survey, and the staff of the Department of Antiquities, who assisted in the excavations. I also thank M. D. Leakey for help, advice and encouragement.

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New cranium of *Homo erectus* from Lake Ndutu, Tanzania

I DESCRIBE here the humanoid cranium recovered by Mturi¹ during excavations at Lake Ndutu in northern Tanzania. A fuller description will be published elsewhere.

The cranium is remarkable in that it seems to form an evolutionary link between *Homo erectus pekinensis* and *Homo sapiens*, having features in common with both. It cannot, however, be classified as *Homo sapiens*, and in spite of its strong resemblance to *H.e. pekinensis* its more advanced characteristics and its occurrence in Africa rather than Asia may eventually warrant the creation of a new subspecies of *H. erectus* to accommodate it. This will be determined following full comparative studies.

The specimen is similar to *H.e. pekinensis* in the following features:

- The form and contour of the occipital, which has a markedly thickened nuchal torus.
- The form of the mastoid region.
- The almost vertical forehead.
- The inferred supraorbital torus.
- The great thickness of the vault.
- The outline, as seen in norma verticalis.

The cranium differs from *H.e. pekinensis* and is similar to *H. sapiens* in the following features:

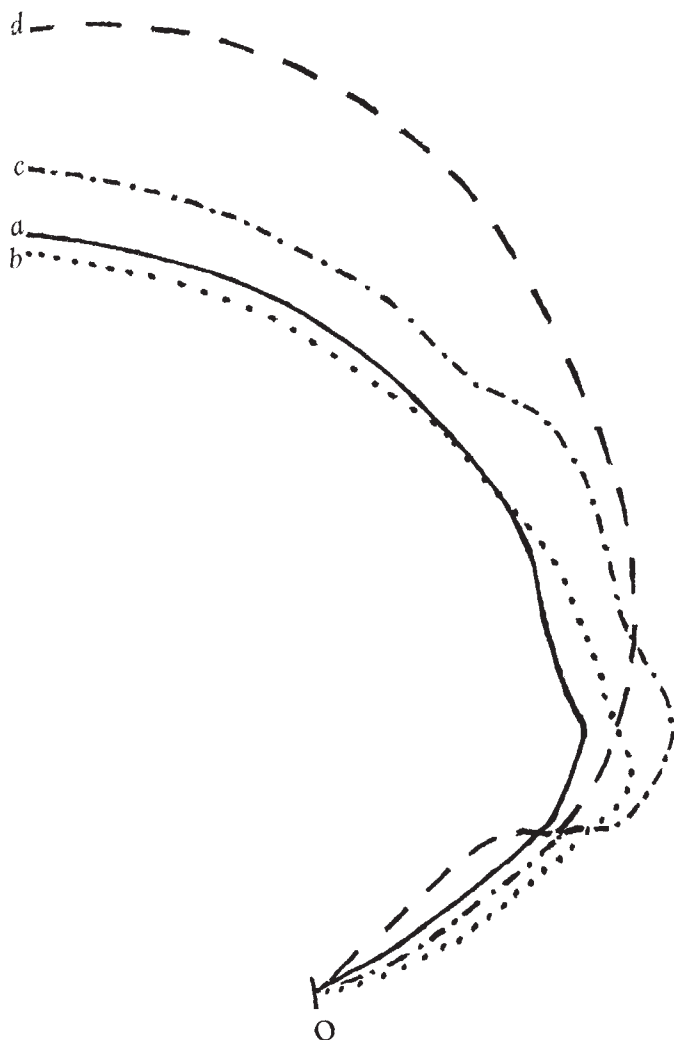


Fig. 1 Mid-sagittal craniograms of the occipital region: a, Ndotu cranium; b, *H.e. pekinensis* (Skull III from locus L); c, Ngandong (Solo), Skull V; d, Australian aborigine; O, opisthion. Adapted from Weidenreich².

- The presence in the adult of pronounced parietal bosses.
- The more vertical sides of the vault as viewed in norma occipitalis.
- The presence of an ossified styloid process.
- The apparent absence of a sagittal torus.
- The supramastoid crest does not extend over the external acoustic meatus.

The Ndotu cranium is housed at the National Museum of Tanzania in Dar es Salaam. The specimen includes the virtually complete occipital and left temporal; the right temporal lacks the glenoid fossa, parts of both parietals and part of the right side of the frontal, parts of the sphenoid, and the fragmentary central region of the face which is attached to a fragment of the left maxilla. In addition, there are isolated right tympanic plates and both isolated petrous temporals.

Before reconstruction the parts of the cranium were very much broken and separated, but were retained in their approximate anatomical position by a matrix of sand and clay which was impregnated with a hardener (Bedacryl) before the cranium was removed from the site. Much of the parietals had suffered badly from cracking and fragmentation, and also from a displacement of inner and outer tables. In parts the inner table only was present and in others only the outer table in the form of a thin shell with the diploë missing. In other places the inner and outer tables had been pushed apart from the diploë, and the spaces between were filled by the sandy matrix, thus giving an artificial, exaggerated thickness to the vault.

The occipital is nearly complete, lacking only the left occipital condyle and some small areas near the foramen magnum. The foramen magnum measures 37.5 mm in anteroposterior length and 28.7 mm in breadth. The apex of the occipital is represented by a sutural bone. There is also a sutural bone to the right of this along the lambdoid suture. On each side there are two small sutural bones at asterion, but one of those on the right had fallen out and was not recovered during the excavation.

There is a well developed nuchal torus as in *H.e. pekinensis*, giving the bone a markedly angulated lateral contour (Fig. 1). The bone is fairly thick, particularly at asterion.

The left of the temporals is the more complete. Its mastoid process is small and of a triangular horizontal cross section. The posterior part of the mastoid is flattened and lies in the nuchal plane. The mastoid is thus similar to that of *H.e. pekinensis*, and is particularly similar to that of Olduvai hominids 9 and 12. There is a well defined mastoid notch which is divided along its length by a central ridge.

An ossified styloid process is present and can be seen particularly well under cover of the isolated right tympanic plate.

The lower margin of the external acoustic meatus preserved on the left is thick and elongated in a downward direction.

A supramastoid crest can be seen, but it does not extend over the external acoustic meatus as in *H.e. pekinensis*.

The left glenoid fossa is deep, relatively small and short anteroposteriorly. The articular tubercle lacks its lateral margin.

The bone at asterion is very thick as is the case in *H. erectus pekinensis*.

The right parietal is missing large areas around its centre and anteromedially, but it has contact laterally at the coronal suture with a frontal fragment. The conformation of the bone around the centre of the parietal indicates that it had a pronounced parietal boss. The parietal thickness at a point anterior to the boss is 11.5 mm. This is well within the upper range of parietal thickness given by Weidenreich² for *H.e. pekinensis*, and much thicker than the range he quotes for *H. sapiens*.

The left parietal is represented mainly by the posteromedial portion externally and the posterolateral portion internally. The anterior part is missing but an anterolateral fragment adjoins the squamous part of the temporal. The left parietal is in articulation with the occipital and left temporal at asterion.

The temporal line is seen only very faintly on the right parietal and, from the contour of the surface of the parietals near the sagittal suture, there was apparently no sagittal torus, such as occurs in *H.e. pekinensis*.

The preserved right frontal fragment is 8.6 mm thick and thus well within the range of 7.0–13 mm given by Weidenreich² for *H.e. pekinensis*, and is at the same time greater than the maximum of 6.3 mm he quotes for *H. sapiens*.

The anterolateral corner of this frontal fragment curves forward and outward, indicating that there must have been a supra orbital torus in order for that fragment to meet with the glabellar fragment attached to the face. The glabella together with the face, could not be placed further back because it is restricted by other anatomical features such as the cribriform plate of the ethmoid bone, and by the necessity for room for the anterior fibres of the temporalis muscle. In reconstruction, allowance was made for only the smallest supraorbital torus permitted by the preserved anatomy, but it is possible that a larger torus could have been present.

The frontal bone rises in a high curve of the forehead as in *H.e. pekinensis* but unlike the frontals of *H. erectus erectus*, the Ngandong crania or the Broken Hill cranium.

The middle portion of the face is preserved, including much of the nasal aperture, the upper part of the left nasal bone, the lower medial parts of the orbital rims together with the lacrimal grooves, and much of the superior part of the right orbital plate. The crista galli is present and is attached to the cribriform plate behind the nasal bones.

On the right, the medial part of the face is preserved as far down as the infraorbital foramen, and on the left there is continuous bone contact from the nasal bone to the palate. The



Fig. 2 *a*, Right norma lateralis of the Ndutu cranium; *b*, left norma lateralis of the Ndutu cranium.

conformation of the bone around the nasal aperture, and the angle between the nasal bones indicate that the nose was very prominent.

The left portion of the palate contains fragments of the roots of the canine, P³, P⁴ and M¹ and the mesiobuccal socket of M². This palatal fragment reaches almost to the midline of the cranium.

Areas of the cranium were reconstructed with plaster of paris both to strengthen the parietal region, where either inner or outer tables only were preserved, and to attach the face to the rest of the cranium. This reconstruction was minimal and preliminary. The reconstruction suggests that slight adjustments can be made to the position of the face, but this will not affect the conclusion that a supraorbital torus must have been present originally. The size and shape of the maxilla remain unknown as it is extremely fragmentary. The top of the cranium has been left open in order that the internal anatomy can be seen.

The cranium (Fig. 2) has some similarities to Olduvai hominid 12 in the thickness of the vault and the shape of the mastoid, but it is much larger. It differs from the Ngandong and Omo II crania in its outline as seen in norma verticalis. The former have a more rectangular outline whereas the Ndutu cranium and *H.e. pekinensis* have a pear-shaped outline. It differs from Ngandong and Omo II also in possessing a more vertical forehead, though it is similar to Ngandong in the morphology of the temporal. The mastoid of the Ndutu cranium differs from that of Omo II. It differs from the Broken Hill cranium in its temporal and frontal and in being much smaller. It differs from Swanscombe and Steinheim in its occipital curvature and in that the mastoid of Steinheim is sapient in form. It differs also from Skhul V in its occipital and mastoid. It bears little comparison with *H.e. erectus* except in vault thickness and in its

outline seen in norma verticalis. The mastoid region is similar to that of Olduvai hominid 9, but otherwise, apart from thickness of the vault, there is little resemblance.

It would have been of interest to compare the Ndutu cranium with that of the Eyassi I cranium³ but unfortunately the original disappeared in Berlin in 1945 according to Weinert³, and available casts are too poor to allow comparison. Published descriptions and photographs are also not of any great help because the cranium was reconstructed from fragments, and it is difficult to ascertain what is reliable. In spite of all of these deficiencies, the Eyassi cranium does seem to have been rather similar to the Ndutu cranium. It was found not far away from Lake Ndutu in a similar deposit on the shore of the saline Lake Eyassi. It had also undergone separation of inner and outer tables of the vault bones, probably as a result of alternating crystallisation and solution of the salt within the diploë. A few broken handaxes and an early water-rolled fauna also occurred at the site, but Leakey^{4,5} considered the cranium to be contemporary with an unrolled modern type of fauna, and with levalloisian artefacts that were also to be found on the surface. The possibility should not, however, be dismissed that the Eyassi cranium could have been contemporary with the hand axes and the rolled fauna. This is supported by the fact that, although Leakey⁴ has described the skull as unrolled, Weinert³ has claimed that the edges broken in antiquity were rounded and rarely fitted together.

The Eyassi II occipital fragment, which is preserved in the National Museum in Dar es Salaam has some similarity to that of the Ndutu cranium.

Finally, it is of interest to note that Dr Peter Andrews subjected the Ndutu cranium to a form of multivariate analysis described by Andrews and Williams⁶. He found that the cranium grouped with *H.e. pekinensis*, thus confirming by measurement the conclusion reached through observation.

I thank the Government of Tanzania and their Director of Antiquities, Amini Mturi, for giving me the privilege of cleaning, reconstructing and studying the Ndutu skull. I also thank Richard and Mary Leakey for providing facilities for the work, the L.S.B. Leakey Foundation for financing my study visit to Nairobi, and Professor P. V. Tobias for making the visit possible.

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Antennal receptor response to sex pheromone mimics in the American cockroach

THE essential oils D-bornyl acetate, α - and β -santalol and several plant sesquiterpene hydrocarbons have been shown to induce sexual excitement in male American cockroaches¹, and thus seem to mimic the cockroach sex pheromone. Tahara *et al.*² have identified one of the active sesquiterpene hydrocarbon species obtained from Compositae plants as germacrene D. The contrast in structures of these various active compounds poses a serious problem as to the specificity of the sex pheromone receptor. To resolve this we have undertaken an electrophysiological study of the antennal receptors of both male and female cockroaches. The electroantennogram (EAG) responses of both sexes were examined during exposure to the compounds in question. Theoretically the sex specific receptors of the male

antennae should respond singularly to natural sex-pheromone (and stereochemical mimics) whereas the female antennae should be unresponsive other than to general irritants and/or to food odours. In fact, the EAG response to the female sex attractant has been obtained in males but not in females^{3,4}.

EAGs were recorded from excised antennae of the adult American cockroach, *Periplaneta americana*. A tungsten electrode, whose tip has been electrolytically sharpened to about 1 μm with resistance of less than 1 M Ω , was inserted into the distal end of the antennae. The electrode was connected to the input probe of a unity gain d.c. pre-amplifier. A wider stainless steel electrode was placed in the scape of the antenna in a ground position. Germacrene D was isolated from the leaves of *Solidago juncea* Ait. by a combination of column chromatography over Florisil and silver nitrate coated Florisil. The spectral data are as follows: ultraviolet (hexane): 222(e6590) and 257 nm (e5020); infrared (film): 3,080, 1,670, 1,635, 1,610, 1,390, 1,375, 1,190, 987, 980 and 890 cm^{-1} ; nuclear magnetic resonance (CCl_4): 0.84(3H, d., $J=6$ Hz), 0.90(3H, d., $J=6$ Hz), 1.53(3H, s.), 4.70 and 4.73(2H), 5.19(1H, q., $J=6$ and 10 Hz), and 5.72 p.p.m.(1H, d., $J=15$ Hz). A test chemical dissolved in acetone was completely absorbed on filter paper with the solvent evaporating at room temperature. The filter paper was placed on a vinyl tubing holder, which had been set in a 2-ml syringe. After 2 ml of air had been drawn into the syringe, the syringe plunger was quickly depressed to mix the odour into an airstream. The air taken from the ambient air supply, filtered through glass wool and distilled water, and passed continually at 60 ml min^{-1} through 5-mm diameter glass tubing. As a control the same procedure was carried out without a test compound.

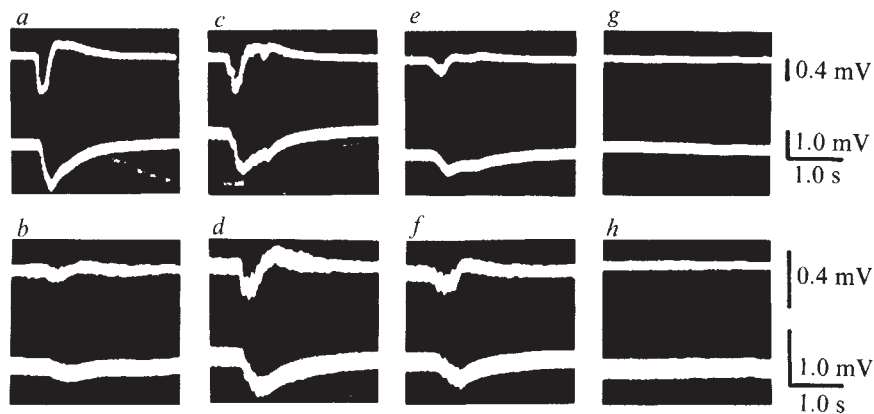


Fig. 1 EAGs recorded from a *Periplaneta americana* adult male (a, c, e and g) and female (b, d, f and h) in response to germacrene D (0.1 mg in a and 3 mg in b), D-bornyl acetate (3 mg in c and d) and L-bornyl acetate (3 mg in e and f). Controls (air without odour) are shown in g and h. In each record upper and lower traces show a.c. and d.c. recording, respectively. Note different calibrations in records of male and female antennae.

Adult male antennae showed strong EAG responses to germacrene D (Fig. 1a). In the female antennae, however, it caused very small negative potentials, even at much higher concentrations (Fig. 1b). The effect of increasing concentration of germacrene D was examined in male antennae. The amplitude of the responses increased in approximate proportion to the logarithm of the intensity of stimulus tested (Fig. 2). The threshold concentration was found to lie between 1 and 5 μg . The response approached a plateau at a concentration of 100 μg or slightly higher. In comparison with EAGs obtained to DL-bornyl acetate⁴ the threshold concentration of germacrene D required to elicit the EAG response was much lower, being of the order of one-hundredth. The result of a single experiment to compare the threshold concentration of germacrene D and D-bornyl acetate showed that 5 μg of the former gave a detectable potential change, whereas 50 μg of the latter did not bring about any EAG response. Although strong EAG responses of male and female antennae to DL-bornyl acetate were obtained in agreement with earlier findings⁴, we examined the effect of pure D- and L-bornyl acetate in the

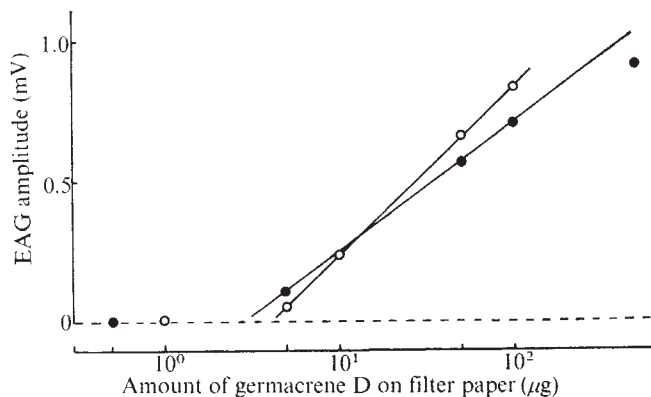


Fig. 2 Amplitude of the EAG responses of two male antennae (○, ●) as a function of the amount of germacrene D (μg) on a logarithmic scale.

present work. The male antennal responses to D- and L-bornyl acetate (Fig. 1c and e) were found to be stronger than those of the female (Fig. 1d and f). Furthermore, both male and female antennae gave stronger EAG responses to D-bornyl acetate than to L-bornyl acetate. Another essential oil, santalol, was found to give very small EAG responses in both sexes.

It has already been shown that strong EAG responses to the female sex pheromone were obtained from the antennae of male adults but not from females^{3,4}. On the other hand, bornyl acetate, which induced significant sexual excitement in the male¹, was found to give strong EAG responses with both male and female antennae. Although adult females show no change in overt behaviour when exposed to bornyl

acetate, they show distinct sensitivity to bornyl acetate in EAG analysis. The response of female antennae to bornyl acetate may be due to the presence of generalised antennal receptors since it is unlikely that bornyl acetate has any natural role in female sexuality. Furthermore, in the present work it was confirmed that only the male antennae responded strongly to germacrene D, while the antennae of both sexes are stimulated by D- and L-bornyl acetate. We were unable to obtain other than minimal EAG responses from either sex with santalol.

Ritter *et al.*⁵ have proposed two sesquiterpenoids for the American cockroach sex pheromone, and have suggested the germacrene skeleton for one of these on the basis of spectral data. We also suggest that germacrene D is closely related to the natural sex pheromone of the cockroach since it induces strong sexual excitement^{1,2}, and shows a sex specific EAG response.

Cadinol, a sesquiterpenoid biogenetically related to germacrene D, has been isolated from a plant and shown to be a stimulant for both male and female cockroaches⁶. This compound together with germacrene D indicates the

importance of sesquiterpenoids in the chemical communication of cockroaches. Lundgren and Bergström⁷ also reported the identification of a cadinol (tentatively identified as δ -cadinol) as a constituent of a *Lepidoptera* wing scent complex, important to courtship behaviour. Thus, cadinol may be significant for other insects.

We thank Fritzsche Dodge and Olcott Inc. for analytically pure samples of D- and L-bornyl acetate, and Givaudan Inc. for α - and β -santalol. We also acknowledge technical assistance from Miss K. Tsuzuki and Miss N. Sekijima.

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Ecdysone and oocyte morphogenesis in *Bombyx mori*

ECDYSONE, one of the principal hormones of insects, controls the periodic shedding and rebuilding of the cuticle during larval development. (We use "ecdysone" to designate all ecdysoids (in the sense of P. Karlson in ref. 5), or, for the radioimmunoassay, all radioimmunoassay-active ecdysteroid compounds.) It also promotes changes in gene activity observed at the initiation of metamorphosis¹ and influences morphogenetic development of the imaginal disks in pupae. Some reports also indicate that ecdysone is found in adult insects^{2–4}. Recent work on *Drosophila* (M.L.D.-R., M. H. Hirn and M. A. Delaage, unpublished), *Tenebrio* (P. P. Delbecque, M. H. Hirn and M.L.D.-R., unpublished), *Locusta*⁵ and termites⁶ has shown that a significant level of ecdysone is generally found in adult insects of both sexes and that high titres of ecdysone can be found in ovarian tissues. In *Locusta*, the ecdysone titre of the ovary increases during each cycle of oocyte maturation⁷, suggesting stimulation by ecdysone, in agreement with observations on *Aedes aegypti*⁸. This reveals an unexpected aspect of the action of ecdysone. Using a radioimmunoassay to study ecdysone action in *Bombyx mori*, we have found that not only is ecdysone present in the *B. mori* ovary during egg production, but that the oocyte itself contains a high concentration of ecdysone. Moreover, this concentration changes during successive phases of oocyte morphogenesis.

In *Bombyx*, oogenesis in a well defined sequence⁹ during pupal development, and is terminated when the insect ecloses. Pupae were used 6, 9 and 15 d after larval-pupal ecdysis (at 22 °C). They were quickly dissected in Ringer solution. For each experiment we removed one ovariole from the right ovary; it was cleaned of adipose tissue fragments and then transferred to a small vial containing a few millilitres of Ringer solution. Oocytes were extracted from the ovarian tube by microdissection. They were collected one after the other, from the bottom to the top of the tube, rank 1 indicating the lowest and therefore the first extracted. Generally, five adjacent oocytes, that is, less than 4 mg wet weight, were sampled for each ecdysone assay. The length and width of the central oocyte of each assay were determined using a micrometer. These measures enabled a good estimate of oocyte volume¹⁰. The samples were then frozen

in liquid nitrogen and lyophilised. Dissection and sample collection did not take longer than 20 min. In these conditions, there was no noticeable loss of ecdysone during collection of oocytes as verified by replicate dissections at various intervals of time. Ecdysone titre was determined in oocytes sampled from the bottom, middle and top of the ovariole. More detailed sampling was carried out on an ovariole from a 13-d-old pupa, to measure precisely the ecdysone gradient along the ovarian tube.

Ecdysone was determined quantitatively by radioimmunoassay, as before¹¹. Lyophilised samples were homogenised in 200 μ l 1 N HClO₄ by brief sonication. The precipitated protein and the insoluble fraction were removed by centrifugation at 15,000g for 5 min. A sample of 150 μ l supernatant was neutralised with 15 μ l 6 M K₂CO₃ and the resulting salts were removed by further centrifugation. A sample of 100 μ l of the second supernatant was used for radioimmunoassay, after suitable dilution in 0.1 M citrate buffer, pH 6.2. Results are expressed as pg β -ecdysone equivalent.

Table 1 Ecdysone content (radioimmunoassay activity) of oocytes in *B. mori* pupae*

Pupal β -ecdysone† (d)		Position of oocytes in the ovarian tube			Total ovariole content (pg β -ecdysone)
		Bottom	Middle	Top	
6	1	1,300	40	ND	21,000
	2	1,870	400	ND	
	3	1.21×0.98	0.60×0.52		
9	1	3,490	2,920	30	123,000
	2	2,810	3,780	460	
	3	1.46×1.21	1.24×1.04	0.53×0.43	
15	1	660	1,170	450	134,000
	2	420	690	1,340	
	3	1.61×1.27	1.62×1.33	0.97×0.75	

*Radioimmunoassay activity is expressed as pg β -ecdysone equivalent.

†1, β -ecdysone equivalent content (pg per oocyte); 2, β -ecdysone equivalent concentration (pg μ l⁻¹); 3, oocyte dimensions: length $\times$ width (mm).

ND, Not done because of small size of oocytes.

Oocytes were collected from the bottom, middle and top of the ovarian tube, in pupae aged 6, 9 and 15 d. Maturation of oocytes begins at the bottom of the ovariole and progresses to the top. To calculate concentration, oocyte volume was estimated by a geometric model¹⁰.

Table 1 shows that maturing oocytes contain ecdysone at high concentrations (the highest value observed was 3,780 pg μ l⁻¹, or 8×10^{-6} M). On day 6, the concentration of the hormone was 1,870 pg μ l⁻¹ in the lowest oocytes, which had just started to mature; whereas there was little or no ecdysone in higher oocytes. Later (day 9) the morphogenetic activity in the ovariole had progressed upwards. The ecdysone titre was now at a maximum in the middle part; it had also increased in the top part of the tube, to 460 pg μ l⁻¹. On day 9, the bottom oocytes had stopped growing; they contained more ecdysone than previously but less than did middle oocytes of the same ovariole. Finally, on day 15, maturing oocytes were the highest oocytes in the ovariole. They had the highest titre of ecdysone (1,340 pg μ l⁻¹), whereas in those at the bottom of the ovariole, where ovulation had finished, the concentration had declined to 420 pg μ l⁻¹. Table 1 also suggests that oocyte maturation leads to an increase in the ecdysone content of the entire ovariole.

Detailed ecdysone assays, on day 13, in oocytes of rank 1 to rank 70 confirm these results (Table 2). The concentration was relatively low in oocytes at the bottom and gradually increased towards the top of the ovariole; it peaked in oocyte 66, in good agreement with the data in Table 1.

Our work demonstrates that maturing oocytes of *B. mori* contain ecdysone, and that ecdysone concentration changes considerably during oocyte morphogenesis. The hormone

Table 2 Ecdysone (radioimmunoassay activity) gradient in one ovariole of a *B. mori* pupa of 13 d

Oocyte rank*	1	16	26	46	56	66	71
Ecdysone content†	830	1,200	1,470	1,770	1,880	2,720	2,250

*Oocytes are numbered from the bottom to the top of the ovariole.
†pg β -ecdysone equivalent per oocyte.

appears at the beginning of maturation and increases during the growth phase, reaching a very high value (close to 10^{-5} M). It then decreases at the end of the process. This lowering of ecdysone concentration is noteworthy; it cannot be attributed to a dilution effect due to oocyte growth, since Table 1 shows that the ecdysone pool in the ovariole continues to increase up to 15 d. This implies a gradient of hormone concentration along the ovariole, showing a maximum which moves from the bottom to the top of the ovariole as oocyte maturation progresses upward. The ecdysone titre peaks at the end of the ellipsoidal phase of oocyte morphogenesis, when the oocyte is about 1 mm wide and starts flattening to take on its final shape^{9,12}.

A few reports have discussed possible roles of ecdysone in ovarian development. In mosquitoes ecdysone controls vitellogenesis¹³; more precisely, it directly activates synthesis of vitellogenin⁸. In the ovary of *Tenebrio molitor*, ecdysone stimulates *in vitro* cell divisions and oocyte growth, and influences follicle organisation¹⁴. Our report of ecdysone in the oocyte itself raises new questions. We propose that ecdysone behaves as a metabolic symbol, that is, a specific intracellular effector molecule which accumulates when the cell is exposed to a certain environment¹⁵. Perhaps it has a double role—as suggested by Laverdure's¹⁴ work *in vitro*—stimulating oocyte growth and influencing oocyte morphogenesis. The ecdysone variation we observed in the ovarian tube could reflect the organisational action mentioned by Laverdure¹⁴. It could also be one element of the longitudinal energy gradient proposed by Legay¹². By our hypothesis, ecdysone action on oocytes is connected to its known effects on the morphogenesis of imaginal disks *in vitro*, in *Galleria*¹⁶ and in *Drosophila*¹⁷.

We were intrigued by the lowering of ecdysone level during the last phase of oocyte development. Further observations indicate that the concentration of the hormone increases again in newly laid eggs of *B. mori* (M.H.H., M. Coulon and M.L.D.R., unpublished). This shows that ecdysone does not simply accumulate in the cell as a passive component, and that specific pathways exist for ecdysone elimination from oocytes.

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Covering response in sea urchins

THE tendency of many species of sea urchins to pick up particles and debris with their spines and tube feet, and to move and then hold the particles on their aboral surface is well known. Functional bases (decreasing the susceptibility to water or wave action, desiccation, light, or predation; supplying nutrients through skin digestion and absorption) have been ascribed to this "covering reaction"^{1–13}.

In contrast to these 'functional' explanations for the covering response of sea urchins, Dambach and Hentschel¹⁴ concluded that the phenomenon is simply reflexive: mechanical, chemical or photic stimuli produce increased tube feet and spine activity involved in locomotion, or in a generally higher activity level, resulting in the covering of the individual. The suggestion that reflexes are involved has been made before^{1,2}, but Dambach and Hentschel are the first to provide experimental data to support it. The functional explanations of weight and protection from light involve stimuli (mechanical and photic) which lead to increased activity of the tube feet and spines^{2,6}. Support for Dambach and Hentschel's proposal also comes from the previously unreported observation that there is a reversal of the covering response by *Lytechinus variegatus* when it is inverted; thus, material held on the aboral surface is quickly moved orally by inverted animals.

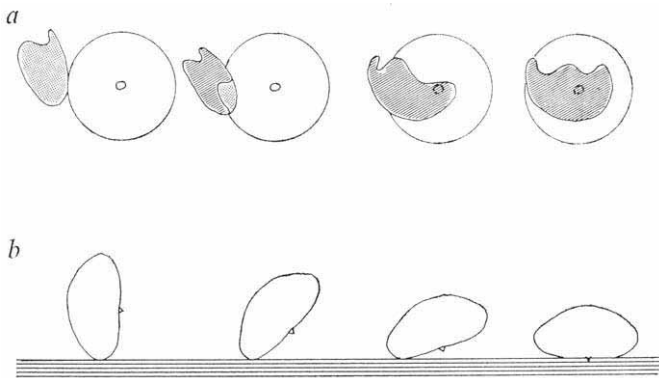


Fig. 1 *a*, Diagrammatic representation of the reversal of the covering response in *Lytechinus variegatus*. A shell, originally on the aboral surface of the animal, has been moved to the ambitus and is subsequently moved to the oral surface. (Drawn from a photographic series). *b*, Diagrammatic representation of the righting response in *Lytechinus variegatus*. The urchin has righted itself to the vertical position where the ambitus is in contact with the substratum. Subsequent positions of the animal in righting correspond to the positions involved in the reversal of the covering response indicated directly above.

Twenty individuals, which were well covered by shell and pebbles on the aboral surface, were collected. The animals were hand held in the inverted position below the surface of seawater in a large container. In each case, the material held by the sea urchin on the aboral surface was moved orally (Fig. 1*a*). This reversal of the covering response seems to be a reflex, particularly when compared with the well known righting response of sea urchins—the tendency of inverted animals to right themselves if physically possible. The activity of the tube feet and spines in the movement of particles in the reversal phenomenon cannot be distinguished from their activity in the righting response; the movement of a particle

over the surface of the animal in reversal is identical to that of the animal's surface over the substratum in righting (Fig. 1b). A reflexive basis for the reversal of the covering reaction supports the suggestion of a reflexive basis for the covering reaction itself. The functional consequences of covering as proposed by other workers could still be valid, but would not be the casual explanations of the phenomenon.

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Testing the neutral mutation hypothesis by distribution of single locus heterozygosity

ONE of the most controversial issues in population genetics at present is whether the widespread protein variation in natural populations is maintained by some form of balancing selection^{1–3} or merely represents the drifting polymorphism of neutral or nearly neutral mutations^{4–6}. By the neutral mutation hypothesis the level of genetic variability in an equilibrium population is determined by mutation rate, ν , and effective population size, N_e . If we use the infinite allele model, in which new mutations are assumed to be always different from the pre-existing alleles in the population, the average heterozygosity per locus is given by

$$H = M/(1 + M) \quad (1)$$

where $M = 4N_e\nu$ (ref. 7).

Whether the observed heterozygosity agrees with the above theoretical value or not is, in practice, however, very difficult to examine, since the exact values of N_e and ν in a population are hardly obtainable. Recently, it was shown^{8,9} that the variance of single locus heterozygosities is given by

$$V(h) = \frac{2M}{(1 + M)^2(2 + M)(3 + M)} \quad (2)$$

From this the neutral mutation hypothesis can be tested without knowing the values of N_e and ν separately. By estimating M from the observed value of average heterozygosity, we can compute the theoretical variance by using equation (2) and then compare it with the observed variance¹⁰. Preliminary results of the application of this method to a number of organisms have suggested that the agreement between the theoretical and observed variances is rather good^{11,12}, but the amount of data used was too small to make any general conclusion. We have accumulated all published data which are amenable to such a test. In this report we show that the observed variances of

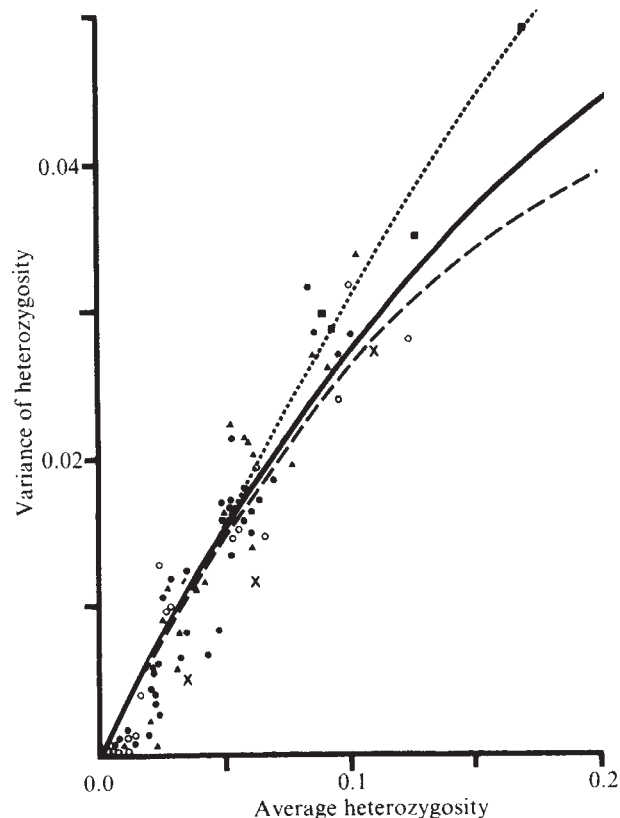


Fig. 1 Relationship between the mean and variance of heterozygosity for vertebrate species: ●, mammals (33 species); ×, birds (2 species, 1 subspecies); ○, fish (18 species, 1 subspecies); ▲, lizards (21 species); ■, amphibians (3 species, 1 subspecies). The solid and dashed lines are the theoretical relationships obtained from the infinite allele and stepwise mutation models, respectively. The dotted line is the theoretical relationship for the infinite allele model with varying mutation rate.

heterozygosity in most organisms so far studied can be reasonably explained by the neutral mutation hypothesis. The pattern of the distribution of heterozygosity also agrees with that expected from this hypothesis.

Surveying 52 publications, we collected gene frequency data obtained by electrophoresis for 123 species and 8 subspecies, in which 15 or more loci were examined for at least 30 genomes. It is known that for estimating average heterozygosity a large number of loci should be used, whereas the sample size per locus can be rather small¹⁰. For each of the species and subspecies used, the mean, $\hat{H}$, and variance, $\hat{V}(h)$, of single locus heterozygosity was computed with a correction for sampling variance¹⁰. When a species or subspecies comprised a number of subpopulations, the average values of $\hat{H}$ and $\hat{V}(h)$ for these were used. The relationship between $\hat{H}$ and $\hat{V}(h)$ was compared with the theoretical one (solid lines in Figs 1 and 2) obtained from equations (1) and (2). It can be argued that the stepwise mutation model¹³ is more appropriate for electrophoretic data than the infinite allele model. Therefore, we have also computed the theoretical curve for this model (broken line) using Ohta and Kimura's formula¹³ for H and Moran's formula¹¹ for $V(h)$. Actually, as Figs 1 and 2 show, there is not much difference between the two models unless H is very large. Since there seems to be a significant difference in average heterozygosity between vertebrates and invertebrates¹⁵, the two groups have been considered separately (Figs 1 and 2).

It is clear that the observed variances for vertebrates are generally in good agreement with the theoretical values, in spite of the fact that the number of loci used is relatively small and data on diverse organisms are included. A close examination of Fig. 1, however, indicates that when $\hat{H}$ is smaller than 0.05

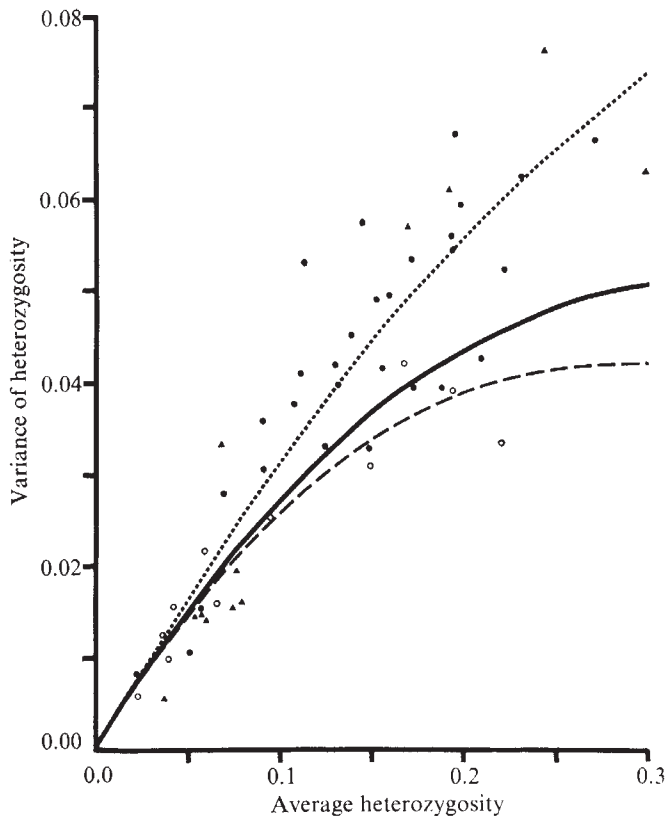


Fig. 2 Relationship between the mean and variance of heterozygosity for invertebrate species: ●, *Drosophila* (24 species, 5 subspecies); ▲, other insects (12 species); ○, other invertebrates (10 species). The solid and dashed lines are the theoretical relationships obtained from the infinite allele and stepwise mutation models, respectively. The dotted line is the theoretical relationship for the infinite allele model with varying mutation rate.

the observed variance tends to be smaller than the theoretical curve, whereas for $\hat{H} > 0.1$ it tends to be larger. The agreement between the theoretical and observed variances in invertebrates is also generally good, though the tendency for the observed variance to deviate upward is pronounced because of higher heterozygosities in this group of organisms.

The tendency for the observed variance to deviate upward for large $\hat{H}$ does not contradict the neutral mutation hypothesis and is expected to occur for the following reason. The formula for $V(h)$ is based on the assumption of identical mutation rates for all loci in both theoretical models used here. In practice, this assumption is surely incorrect, and when mutation rate varies among loci, the variance of heterozygosity is expected to inflate. To get an idea about the amount of inflation, we computed the expected variance of heterozygosity from the assumption that mutation rate varies among loci according to the gamma distribution with the coefficient of variation equal to one. The gamma distribution with this value of coefficient of variation was suggested by the examination of the rates of amino acid substitution for 19 polypeptides in evolution (Nei, Chakraborty and Fuerst, unpublished). From the assumption of neutral mutations the rate of amino acid substitution per polypeptide is equal to the mutation rate per locus, neglecting synonymous mutations⁴. At any rate, the results obtained are given by the dotted lines in Figs 1 and 2. It is clear that the agreement between the theoretical and observed variances is now very good.

The tendency for the observed variance to deviate downward for a small value of $\hat{H}$ is apparently caused by the relatively small number of loci studied (about 20) in this group of organisms. When the theoretical value of H is relatively small, the

distribution of single locus heterozygosity, h , is extremely skewed towards the class of $h = 0$ (Fig. 3). Thus, whenever the sample estimate of H is small, the observed value of $V(h)$ tends to be smaller than its expected value. Our computer simulation has shown that the difference between the observed and theoretical variances for $\hat{H} \leq 0.05$ is of the order of magnitude that is expected to occur when the number of loci is about 20. The details of this study will be published elsewhere. Thus, the data presented in Figs 1 and 2 are consistent with the neutral mutation hypothesis.

In Fig. 3 the frequency distributions of single locus heterozygosity for three species groups in which a large number of loci have been studied are presented together with one theoretical distribution obtained by computer simulation¹⁰. This theoretical distribution is based on 2,000 replicate evaluations of h with the expected heterozygosity of 0.09, which is close to the observed average heterozygosities for the three species groups. All empirical distributions are L-shaped and very similar to the theoretical one. In both empirical and theoretical distributions there is a little peak around $h = 0.5$. Existence of this peak has been theoretically predicted^{8,17}. In addition to those in Fig. 3, we examined the distribution of heterozygosity for all other species. The pattern of the distribution was essentially the same as that expected from the neutral mutation hypothesis for most species.

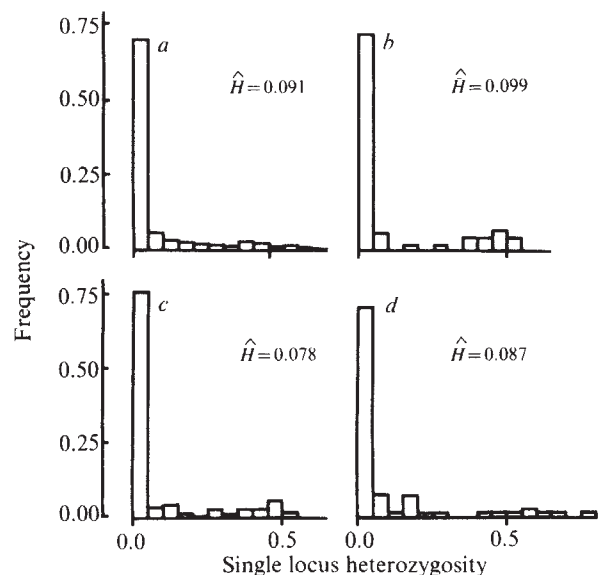


Fig. 3 Distributions of single locus heterozygosities for *b*, man (Caucasian)¹¹; *c*, the house mouse^{20,21} (*Mus musculus*) and *d*, the *Drosophila mulleri* species group²². The theoretical distribution (*a*) was obtained by computer simulation with the infinite allele model. $\hat{H}$ stands for the average heterozygosity. The distribution for the stepwise mutation model is almost identical with that for the infinite allele model when $\hat{H} = 0.09$.

We are aware that our observations about the variation and distribution of heterozygosity can be explained by some elaborate combination of different types of selection. Such an explanation is, however, strained, since we then have to assume that selection is adjusted so as to have a good agreement between the observed and theoretical variances in diverse organisms. It seems to us that the simplest and most natural explanation is the neutral mutation hypothesis. Note that this hypothesis does not exclude the existence of a small proportion of overdominant or selectively advantageous mutations. Furthermore, our analysis will not exclude the possible importance of slightly deleterious mutations^{18,19}, of which the contribution to heterozygosity is relatively small.

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Y chromosome visibility in quinacrine-stained human spermatozoa

THE fluorescent dye, quinacrine, binds strongly to the Y chromosome and only to a lesser extent to the other chromosomes in human metaphase cell preparations¹. A bright fluorescent spot (F body), which is presumed to be the Y chromosome, is clearly visible in stained interphase cells from various tissues from the human male^{2–5} and in mature spermatozoa^{6,7}. Spermatozoa bearing one F body (about 45% of the total) are taken to be Y spermatozoa, and the staining method is now being used widely to detect physical differences, for example, in DNA content and head size^{7,8} and motility⁹ between X and Y spermatozoa, and for testing the efficiency of procedures designed to separate them^{10–13}. Spermatozoa bearing two F bodies (about 1.3% of the total) have been interpreted as YY spermatozoa^{7,8}, and the relatively high number of such spermatozoa has led to speculation concerning the seemingly high non-disjunction rates during spermatogenesis^{8,14,15}. We here examine the factors which affect Y chromosome visibility in sperm heads, and conclude that the F body count depends critically on observer performance, that the presence of YY spermatozoa cannot be demonstrated decisively using the quinacrine stain, and that measured physical differences between spermatozoa with and without an F body may partly reflect a dependence of the Y chromosome detection efficiency on the size of the sperm head.

When a brightly fluorescent chromosome inside a nucleus is examined under the ultraviolet microscope it appears as a bright spot against a less bright background which is produced by fluorescence from the other nuclear contents and from any unbound dye which may be present. The chromosome is detected by the observer provided that the spot intensity is more than 1 or 2% above that of the background¹⁶, the condition for detection then being

$$(I_0 \exp(-k\bar{x}) - I_B)/I_B \gtrsim 0.01-0.02 \quad (1)$$

where $\bar{x}$ is the mean depth (weighted and averaged over all directions which lie within the acceptance cone of the objective lens) at which the chromosome is buried in the head, I_0 is the

intensity of the spot which would be produced if the chromosome were located at $\bar{x} = 0$, I_B is the background intensity and k is an optical attenuation coefficient.

Almost 50% of spermatozoa are expected to carry a Y chromosome, and since only about 45% exhibit an F body it seems that about a tenth of all Y chromosomes are not detected by the staining procedure. There are two possible explanations. The first is that stain uptake among Y chromosomes from cells of any one individual may be very variable, the missing chromosomes being those which absorb so little stain as to be indiscernible above the background fluorescence. The second is that the optical attenuation coefficient is sufficiently great that chromosomes lying deep in the head are not detected⁶. This coefficient has two components. The refractive index of the DNA within the head is high (probably about 1.5 (ref. 17)) compared with that of the aqueous mounting medium (1.33) which surrounds and permeates the sperm head. If the fluorescent Y chromosome is located at the bottom of a uniform sphere of refractive index 1.5 immersed in a medium of refractive index 1.33, the average refractive deviation of light leaving the chromosome and passing through the sphere is found using Snell's law to be about 15°, sufficient to reduce the intensity of a chromosome of diameter 500 nm to about 25% of its undiminished value. If the other chromosomes each refract the light from the Y chromosome in this way the total deviation will be considerably greater than 15° across the optically inhomogeneous sperm head. In addition, the dimensions of the chromosomes are comparable with the wavelength of the fluorescent light, and diffractive scattering will occur within the sperm head, increasing background fluorescence at the expense of spot intensity. Optical attenuation may therefore be the dominant mechanism, and some support for this hypothesis is afforded by the observation that about 70% of F bodies appear at or very near the region where the background fluorescence becomes less dense¹⁵. We have estimated the intensities of F bodies by comparing microscope images of quinacrine-stained spermatozoa with matching images projected on a screen in the same conditions (green light; dark-adapted vision) and measuring the screen intensities with a photometer. F body intensities varied from about 10% (the brightest) to about 1% (that is, the detection threshold) above background. Assuming that the brightest spots correspond to Y chromosomes at $\bar{x} = 0$ and that the Y chromosome is randomly distributed within the head, then $k \sim 50 \text{ mm}^{-1}$ would give the observed proportion of spermatozoa carrying an F body.

Other chromosomes, although fluorescing less brightly than the Y chromosome, may nevertheless be detected if they are located near the surface of the sperm head. If P_y is the probability of detecting a Y chromosome in a Y spermatozoon, and P_c is the probability of detecting any other chromosome (or other artefact such as adhering bacteria or vacuoles within the head¹⁵) then, assuming that X and Y spermatozoa occur in equal numbers (and, initially, that no YY spermatozoa are present) the percentages of spermatozoa exhibiting one and two F bodies, P_1 and P_2 , are given by

$$P_1 = 50P_y(1 - P_c) + 100(1 - \frac{1}{2}P_y)P_c$$

and

$$P_2 = 50P_yP_c + 100(P_c)^2$$

Solving for $P_1 = 45\%$ and $P_2 = 1.3\%$ yields $P_y \sim 0.89$ and $P_c \sim 0.0275$, this being the only solution for which both P_y and P_c lie within the range 0–1. These figures may be compared with those from buccal mucosa cells and lymphocytes where cells of known karyotype are examined^{2,3}. A fluorescent spot indistinguishable from a normal Y body² occurs in about 5.5% of cells from 46,XX females who carry no Y chromosome, and cells from 46,XY males, carrying one Y chromosome, have one F body in about 67% of cases^{2,3}. Thus $P_y \sim 0.65$ and $P_c \sim 0.055$, and the Y chromosome is clearly more difficult to detect in these cells than in spermatozoa.

Several conclusions may be drawn from this analysis. First, if the artefact rate (P_c) in spermatozoa is comparable with that in somatic cells it is clearly unnecessary to invoke the presence of any YY spermatozoa to account for those which exhibit two F bodies. It therefore seems that no estimate of the non-disjunction rate for the Y chromosome can be obtained from staining data, and similar considerations probably apply to estimates obtained using differential stains which are specific for other chromosomes⁸. If YY spermatozoa are not present in appreciable numbers then about 90% of Y spermatozoa are detected by the technique, and of those with one F body about 97% are Y spermatozoa, the rest being X spermatozoa with another fluorescing chromosome or other artefact. These figures for the efficiency may be optimistic, however, since it is assumed that the criterion for detecting an F body is the same whether one or two are present in the cell, and it seems likely that spermatozoa are only classified as bearing two F bodies when both are reasonably bright¹⁵.

Second, equation (1) shows that the counting rate depends critically on observer performance. An observer who decreases his threshold from, say, 2% (distinct spots) to 1% (where any slight local brightening of the background is taken to be an F body) will, on the basis of the present model, increase his F body count from 40 to 50% (with only 40% of the increase coming from newly detected Y chromosomes); and the increase will be even greater if other chromosomes become visible at the new threshold level.

Finally, the present theory suggests that Y chromosome visibility depends on sperm head size, and on the basis of a constant optical attenuation coefficient of 50 mm^{-1} we estimate that P_y will increase from 0.8 to 1.0 with decreasing head size over the range of naturally occurring head volumes¹⁸, together with a corresponding variation in P_c . This may account for the higher detection efficiency in spermatozoa compared with that in the larger somatic cell nuclei. Larger Y spermatozoa will therefore tend to be categorised as X spermatozoa, although the effect could be reduced (or even reversed) if for some reason the background fluorescence of the head itself decreased with increasing head size. Unexpectedly large differences in head area between spermatozoa with and without an F body⁸ may therefore reflect variations in chromosome visibility with head size, and need not necessarily imply the occurrence of haploid gene expression in spermatozoa.

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Absence of axonemal arms in nasal mucosa cilia in Kartagener's syndrome

A TOTAL lack of axonemal arms in the spermatozoa of infertile men has been described¹⁻³ and those results included those from four men, three of whom had situs inversus of the thoracic organs while the fourth was a brother to one of the others. The total absence of axonemal arms (dynein arms) was deemed to be responsible for the complete immobility of their sperm tails. Kartagener's syndrome⁴ exhibits the following symptoms: chronic sinusitis, bronchiectasis and situs inversus. Sperm flagella and cilia have similar ultrastructure in principle, so it was thought possible that the respiratory tract symptoms in patients with Kartagener's syndrome might be caused by a lack of dynein arms in the cilia. We have thus investigated the cilia of Kartagener patients at the substructural level.

The patient examined was a 25-yr-old woman, who had had daily airway symptoms from birth consisting of a cough and mucopurulent secretion from the lower, as well as from the upper, respiratory tract. She underwent surgical treatment for sinusitis, nasal polyposis and bronchiectasis. Her hearing was also impaired by chronic secretory otitis media. X-ray examination demonstrated dextrocardia. Rhinoscopy revealed small nasal polyps, but patent nasal airways. Nasal mucociliary transport function was examined on three separate occasions using the saccharine-dye method⁵. After cocaine anaesthesia, a mucosal biopsy was taken from the inferior turbinate. An additional biopsy from a polyp was taken without anaesthesia. The tissue was immediately fixed in collidine-buffered glutaraldehyde and after a short buffer rinse, postfixed in osmium tetroxide with the same buffer. After dehydration in graded concentrations of ethanol and propylene oxide the tissue was embedded in Epon. Sections stained with uranyl acetate and lead citrate were examined in a Philips 301 electron microscope. The technique was identical to that used for the examination of immobile human spermatozoa^{1,2}.

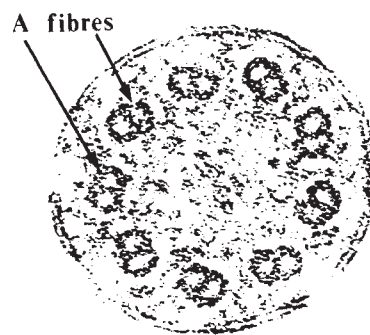


Fig. 1 Cross section of a cilium showing derangement of the axonemal matrix, but normally placed outer doublets. There is no consistent structure comparable with dynein arms on the A fibres. In this section the two central tubules are not recognisable but in other sections they seem to be normal.

After intranasal application of the saccharine-dye particle, the patient was observed for at least 2 h, during which she did not notice any sweet taste in the mouth and no dye was observed in the throat. The normal mucociliary transport time in the nose is 10–30 min (ref. 5). Electron microscopy showed that the cilia had a uniform appearance and transverse sections revealed identical pathological findings. The microtubules were normal in position and appearance, but a common abnormal feature was an apparently total lack of dynein arms on the outer microtubules. The picture was obscured to a certain extent by complete disorganisation of the axonemal matrix (Fig. 1), so that only occasional

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remnants of the central sheath and spokes could be recognised. Many cross sections of cilia were examined and all showed the same abnormal features. One single cilium had structures similar to dynein arms, but whether or not these represented remnants of the C-tubule of the centriole could not be determined. All cross sections of centrioles seemed to be normal.

There is significant experimental evidence that dynein arms are essential for ciliary and flagellar motility, possibly by containing, or consisting of, protein with contractile properties (for review, see ref. 6). Spermatozoal motility is apparently not possible where there is a total lack of axonemal arms¹⁻³. Our results suggest that these structures are also necessary for ciliary motility in the airways, as experiments have shown total absence of bronchial mucociliary transport in patients with Kartagener's syndrome⁷. Chronic infection, various pharmacological treatments and the preparatory procedures might, however, obscure the original basic defects and further studies, including more control measures, are needed.

If the present results and those described previously in human spermatozoa indicate the inability of such patients to produce dynein arms the same changes should be found in cilia from other sources, such as endometrium and uterine tubal mucosa. Such studies, including all the control measures mentioned above, are in progress in this laboratory. The patient described here has a long standing primary infertility of unknown origin.

Although Kartagener's syndrome simulates an immunodeficiency, immunological investigations have failed to disclose any factors of primary importance⁸. An hereditary disability to form dynein arms and the consequent lack of any mucociliary function could, however, explain the symptoms in these patients, and also why they are localised to ciliated organs.

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Separation of suppressor and killer T cells by surface phenotype

AMONG thymus-derived (T) lymphocytes of the mouse, several functionally distinct subsets may be identified (for review, see ref. 1). Early studies have shown synergy in graft-versus-host reactions of different populations of lymphoid cells², and *in vitro* studies have demonstrated interactions between cells from anti-lymphocyte serum-treated mice (T₁ cells) and adult thymectomised mice (T₂

cells)³. A similar synergy has been observed in the generation of T helper and T suppressor cells *in vitro*⁴. Clearer definition of the subsets of T cells interacting in the induction of immune responses has come from the use of alloantisera recognising differentiation antigens on T cells. In particular, the use of antisera to the Ly series of antigens⁵ has enabled the separation of amplifying (MLR) cells from killer cells⁶. Similarly in the humoral response helper T cells can be distinguished from suppressor T cells⁷. In this report, we show that killer and suppressor T cells, both generated *in vitro* and carrying the Ly-2 and 3 antigens can also be separated using antisera to surface antigenic markers. We also present data suggesting that suppressor cells of similar phenotype may have an important function *in vivo* in the immune response.

Soluble protein antigen-specific suppressor cells and alloimmune killer cells were both generated in Marbrook culture flasks, as described previously^{8,9}. Keyhole limpet haemocyanin (KLH)-specific suppressor cells were assayed in a second culture by their ability to abrogate the effect of KLH-primed helper cells^{8,10}. Killer cells were assayed in a 4-h ⁵¹Cr release test on P815 mastocytoma cells¹¹.

Anti-Ly antisera were prepared and tested as described previously^{7,12}. Anti-Ia^k was prepared by repeated intraperitoneal immunisation of A.TH mice with A.TL spleen cells¹³, and used unabsorbed. Rabbit serum absorbed with mouse spleen cells was the source of complement.

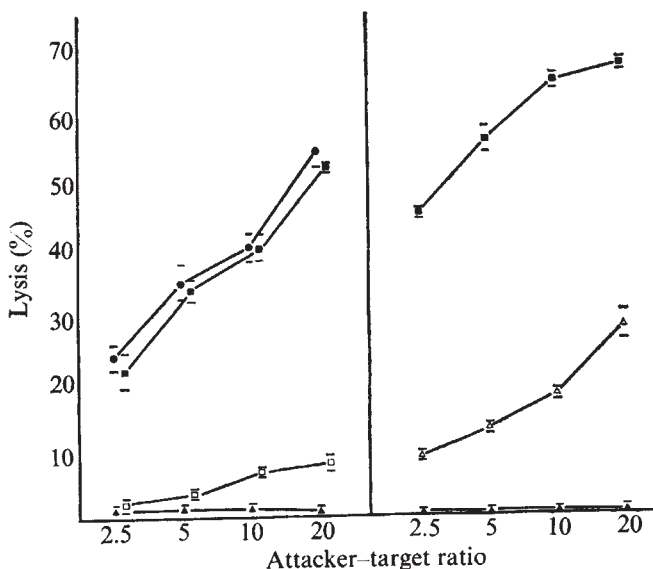


Fig. 1 Effect of anti-Ly, anti-Ia^k and anti-Thy-1 antisera and complement on alloimmune killer T cells. CBA killer cells were induced in Marbrook culture flasks by stimulation of spleen cells with 1,500 R irradiated BALB/c spleen cells⁹. Aliquots of 2×10^6 viable cells from the cultures were treated with 0.2 ml antiserum (at a concentration giving maximal lysis⁷) washed and treated with 0.2 ml absorbed rabbit complement at 1:10 dilution. After treatment cells were washed and resuspended to a fixed volume, giving a viable cell concentration in medium or complement-treated aliquots, of 2×10^6 cells ml⁻¹. Aliquots (0.1 ml) of treated cell suspensions were added to wells of round bottom microtitre plates (Linbro), containing 0.1 ml of a suspension of ⁵¹Cr-labelled P815 mastocytoma cells at 10^5 cells ml⁻¹. After brief centrifugation at 150g (approximately 1 min) the plates were incubated for 4 h in a moist atmosphere of 10% CO₂ in air. After a further centrifugation (300g for 1 min), 0.1 ml supernatant was collected from each well for γ counting. Low controls were incubated in medium (HEPES buffered Dulbecco's Eagles medium) alone and 100% ⁵¹Cr release was taken as counts released by 5% Brij. ●, Anti-Ia^k; □, anti-Ly-2.1+3.2; ▲, anti-Thy-1.2; △, anti-Ly-1.1; ■, complement alone. Sera in absence of complement had no effect (data not shown). Four experiments with anti-Ly-1.1 and five with anti-Ia^k have been carried out using three batches of antiserum. All points are mean of triplicate cultures; bars represent standard error; % lysis calculated by the formula: (c.p.m. killer cells - c.p.m. medium alone)/(c.p.m. detergent) $\times$ 100

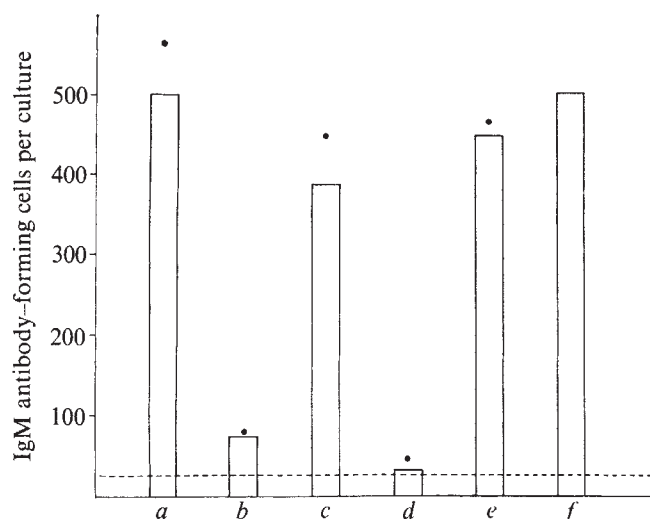


Fig. 2 Effect of anti-Ia^k and anti-Ly-3.2 sera and complement on specific suppressor cells generated *in vitro* from CBA spleen cells. Helper cells were induced as described previously¹⁰. In brief, spleen cells were cultured in Marbrook flasks with 0.1 µg KLH (a gift of Dr M. B. Rittenberg, Portland, Oregon) for 4 d in Eagle's MEM containing 5% foetal calf serum. Helper cells were assayed, in a second Marbrook culture, by their ability to cooperate with trinitrophenol (TNP)-reactive B cells present in normal CBA spleen in the presence of TNP-KLH. Anti-DNP or -TNP antibody-forming cells were assayed using hapten-conjugated anti-sheep red blood cell (SRBC) Fab' fragments coupled to SRBC, using the Cunningham plaque assay. Suppressor cells were induced by culturing in similar conditions but with 100 µg KLH. Suppressors were assayed by their capacity to inhibit the helper effect of 3×10^5 helper cells. Tests for the specificity of the suppressor effect were as described previously⁸. Suppressor cells were treated with anti-Ia at 1:10 dilution or anti-Ly-3.2 at 1:20, washed and treated with absorbed rabbit complement at 1:10. After treatment cells were counted in Trypan blue and 10^4 – 10^6 viable cells assayed for suppressor activity. In absorption experiments sera were absorbed for 30 min at room temperature with an equal volume of packed spleen and thymus cells of appropriate mouse strain (A.TL or A.TH) and diluted to 1:10 for use. Dotted line, Number of plaques produced in presence of antigen but absence of helper cells. Dots above bars, s.e. a, Helper cells; b, helper cells+suppressor cells; c, complement+anti-Ia; d, complement+anti-Ia (absorbed using A.TL); e, complement+anti-Ia (absorbed using A.TH); f, complement+anti-Ly 3.2.

Figure 1 presents the results of two typical experiments on CBA (H-2^k, Thy-1.2, Ly-1.1, 2.1, 3.2) killer cells. In both experiments anti-Thy-1.2 completely abolished cytotoxicity, whereas anti-Ia^k had no effect. Anti-Ly-1.1 and the mixture of anti-Ly-2.1+3.2 both substantially reduce killer cell activity. In similar experiments anti-2.1 and anti-3.2 reduced but never abolished cytotoxicity, the effect of an individual serum being less than the mixture. In contrast to these results, Fig. 2 shows the effects of some of the same antisera on suppressor activity. Anti-Ia abolished suppressor activity, whereas anti-Ly-1.1 had no effect up to $1/10^7$. We have previously shown that anti-Ly-2.1 alone completely abrogates suppressor activity⁷; Fig. 2 shows that the same is true for anti-Ly-3.2.

That the effect of the anti-Ia antiserum was due to activity against antigenic determinants coded by the *I* region, was confirmed by the results of absorption experiments (Fig. 2). The anti-CBA suppressor activity could be removed by absorption with A.TL lymphocytes, which have the same H-2^k *I* region as CBA, but not by A.TH cells which are H-2^s. Note that the serum was also able to abolish the suppressor activity of C57BL/10 cells (data not shown) which share with CBA only a single known *I* region specificity¹⁴.

Table 1 presents an *in vivo* correlate of the *in vitro* data. KLH-primed T cells, which are able to cooperate with B cells *in vivo* to give an anti-hapten response, gave a greatly increased response after treatment with anti-Ia and complement, indicating that the treatment had removed a population of suppressor cells bearing the Ia determinant.

We conclude that the antigenic phenotype of CBA killer T cells is Ly-1⁺,2⁺,3⁺,Ia⁻, whereas suppressor cells are Ly-1⁺,2⁺,3⁺,Ia⁺. The Ly phenotype for killer cells differs from that found in C57BL mice (Ly-1.2, 2.2, 3.2) in which killer cells are Ly-1⁺,2⁺,3⁺ (refs 6 and 16), but agrees with the finding that in the C57BL Ly-1.1 congenic mouse, killer cells seem to be Ly-1⁺,2⁺,3⁺ (ref. 16). In the same study, anti-Ly-2.1 alone had little effect on killer cells. Our data do not distinguish between the possibility that some CBA killer cells bear either 2.1 or 3.2, or alternatively that all killer cells bear low concentrations of both antigens, but it seems likely that there is heterogeneity among killer cells.

Table 2 Ly phenotype of T cell subsets

Function	Ly phenotype	Reference
Helper	1 ⁺	7, 21
Suppressor	2 ⁺ 3 ⁺	7, 22, 23
Killer in Ly-1.1 mice	1 ⁺ 2 ⁺ 3 ⁺	6, 16
in Ly-1.2 mice	2 ⁺ 3 ⁺	6, 16

It is also clear that although anti-Ly sera are useful markers for T cell subsets, antisera to the two alleles of each locus do not always have the same effect. The reason for this discrepancy is not yet clear but may reside in undetected residual genetic heterogeneity in the Ly congenic stocks or in the differences in titre of the various sera (discussed in ref. 16). Table 2 summarises current information on the Ly phenotype of T cell subsets.

Our findings confirm a previous report that suppressor

Table 1 Reduction of suppressor cell activity by treatment with anti-Ia serum and complement

DNP-primed cells	KLH-primed helper cells	Treatment	Antigen	Anti-DNP response (PFC/spleen)
10×10^6	Nil	Nil	50 µg DNP KLH	$3,660 \pm 660$
10×10^6	2×10^6	Nil	50 µg DNP KLH	$9,240 \pm 686$
10×10^6	2×10^6	C' alone	50 µg DNP KLH	$9,720 \pm 362$
10×10^6	2×10^6	Anti-Ia + C'	50 µg DNP KLH	$26,340 \pm 2,882$
10×10^6	2×10^6	Anti-Thy-1.2 + C'	50 µg DNP KLH	$3,480 \pm 595$

CBA mice (70–200-d old) were used. Mice were primed to dinitrophenol (DNP) by intraperitoneal immunisation, 6 weeks before use, with 100 µg alum-precipitated DNP-chicken γ globulin. KLH helper cells were prepared by two intraperitoneal immunisations with 100 µg KLH absorbed to Bentonite at weekly intervals and subsequently boosted with 100 µg soluble KLH. Five days after the last injection a spleen cell suspension was prepared and passed over nylon wool columns to remove B cells¹⁵. Effluent cells were treated with anti-Ia^k or anti-Thy-1.2, followed by complement C'. Treated cells were mixed with DNP-primed cells and injected into 750 R irradiated CBA hosts. These were boosted intraperitoneally 1 d later with 50 µg DNP-KLH. Seven days later the spleens were removed and assayed for IgG DNP plaque-forming cells.

cells bear Ia determinants¹⁷, and provide new information that killer cells are Ia negative. The finding of Ia on suppressor cells is particularly interesting in view of recent data showing that specific suppressor factor isolated from T cells, carried similar antigens¹⁸. Although some workers have failed to detect Ia antigens on T cells¹⁹, others have reported that both thymocytes and peripheral T cells carry these antigens²⁰. Our data using functional assays for T cells show that at least suppressor T cells carry antigen(s) coded in the I region of the H-2 complex. It is not clear whether exactly the same Ia antigen(s) was detected on B and T cells.

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Plasma membrane glycoprotein which mediates adhesion of fibroblasts to collagen

DEFECTS in cell behaviour associated with malignancy may be causally related to alterations in the plasma membrane of the cell on transformation¹. Much interest has focused on a protein found on the surface of normal but not transformed cells^{2–7}. This protein, designated SF (ref. 5), LETS (ref. 8), band 1 (ref. 9), LI (ref. 3) or CSP (ref. 10), is a glycoprotein^{7,9,11} with a molecular weight of 210,000–250,000 (refs 7, 8 and 12). It is present in the serum¹² and is sensitive to removal from the surface of normal fibroblasts by low levels of trypsin^{2,3,9}. Several biological properties of this protein have been described. Normal fibroblasts arrested in mitosis lack this protein^{8,9}. As normal cells approach confluency, there is a quantitative increase in the amount of this protein present on the cell surface^{8,9}.

Table 1 Attachment of PyBHK cells to collagen in the presence of added factors

Protein addition	Concentration of added protein	% PyBHK cells attached to collagen
None	—	8
Serum (%)	1	50
	10	90
	50	96
	50.5	3 (0–6)
CAF (μg ml ⁻¹)	1	4 (2–7)
	5	9 (7–12)
	10	12 (10–15)
	20	29 (25–32)
	30	71 (65–77)
	40	90 (86–93)
	80	96 (94–97)
PyBHK extract (μg ml ⁻¹)	100	5

Assessment of cell binding to collagen-coated dishes in the presence or absence of CAF was carried out essentially as described by Klebe¹⁴. Plastic Petri dishes (60-mm diameter) were evenly covered with 1.5 ml 0.25% collagen (acid soluble, Type IV, Sigma) in 0.2% acetic acid plus 0.002% phenol red. Dishes were placed in plastic containers, containing open dishes filled with NH₄OH and the containers were sealed for 10 min to allow for gelation of the collagen. The dishes were air-dried overnight, washed with 8 M urea for 20 min then three times for 5 min with H₂O, and allowed to air-dry. The treatment with urea decreased attachment of cells in the absence of added protein and increased the effectiveness of CAF-mediated attachment. Coated dishes were incubated with either 0.5 ml medium, calf serum, or CAF at various concentrations for 1 h at 37 °C. All solutions contained 200 μg ml⁻¹ BSA. During this incubation, cells were removed from monolayers with 0.25% trypsin-EDTA (Gibco), washed twice with medium containing 200 μg ml⁻¹ BSA, and counted in a Coulter counter. Incubated dishes then received 10⁶ cells in 4.5 ml E4-BSA. After incubation for 1.5 h, unattached cells were removed by decanting the medium and washing gently once with fresh medium. Attached cells were removed by treatment with trypsin-EDTA and cells counted in a Coulter counter. The percentage of cells attached to the collagen was calculated as the number of attached cells/total number of attached cells plus the number of unattached cells. Usually 80–90% of plated cells were recovered. The average of five determinations and the range of values are given. Protein was determined by the method of Lowry et al.¹⁹.

whereas confluent normal cells, stimulated by the addition of serum, have lowered levels⁸. In conflict with these results, implying a growth regulatory function, is the finding that stimulation of growth-arrested normal cells by thrombin leads to thymidine incorporation without a concomitant decrease in the levels of this protein¹³. We now present evidence which points to a functional role for the protein as a mediator of the adhesion of fibroblasts to collagen. The protein is probably identical to a serum factor previously reported to be necessary for the attachment of SV40-transformed 3T3 fibroblasts to collagen-coated dishes¹⁴. Based on this functional role, we propose the name cell adhesion factor (CAF) for this protein.

The BHK 21/C13 hamster cells and a derivative transformed by polyoma virus (PyBHK) were obtained from stocks at the Imperial Cancer Research Fund, London, and were maintained in Dulbecco's modification of Eagle's medium (E4) containing 10% calf serum (Gibco) in a 5% CO₂ incubator at 37 °C. The BHK cells possess high

Table 2 Ca²⁺ and Mg²⁺ dependence of cell attachment to collagen in the presence of CAF

Cell	CAF (μg ml ⁻¹)	Ca ²⁺ /Mg ²⁺ *	% Cells attached†
PyBHK	—	—	5
PyBHK	100	—	9
PyBHK	—	+	8
PyBHK	100	+	93

* Assayed in PBS (Gibco) in the presence or absence of CAF or in PBS containing Ca²⁺ and Mg²⁺ (Gibco) with or without CAF as described in Table 1 and ref. 14.

† Average of two determinations.

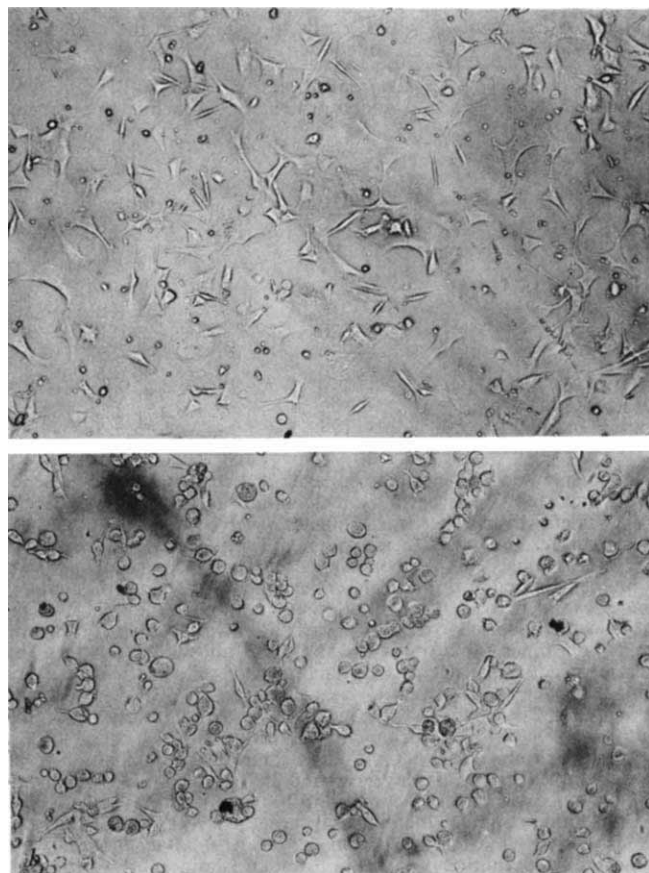


Fig. 1 Attachment of PyBHK cells to collagen-coated dishes under the influence of CAF (magnification $\times 65$). Cells were incubated 1.5 h at 37 °C with 80 $\mu\text{g ml}^{-1}$ CAF (a) and media containing 200 $\mu\text{g ml}^{-1}$ BSA (b).

levels of CAF, whereas the PyBHK cells lack detectable amounts⁹.

CAF was purified from BHK cells as described previously¹³. Polyacrylamide gel analysis demonstrated that the major purified protein co-migrated with the lactoperoxidase iodinated CAF obtained after surface labelling of BHK cells⁹. Immediately before use in the attachment assay, purified CAF was solubilised in medium containing 200 $\mu\text{g ml}^{-1}$ BSA.

BHK-extracted CAF was effective in mediating the attachment of cells to collagen-coated dishes. The results

shown in Table 1 clearly indicated a dose-dependent attachment to collagen in the presence of CAF. In agreement with others¹⁴, no attachment was seen in medium containing only 200 $\mu\text{g ml}^{-1}$ BSA. In optimal conditions, 96% of recovered cells had attached to the dishes. Table 1 shows that half-maximal attachment occurred at approximately 25 $\mu\text{g ml}^{-1}$ CAF. Extracts of PyBHK at 100 $\mu\text{g ml}^{-1}$ failed to enhance attachment of cells to the collagen.

The photographs of cells in Fig. 1a demonstrate that cells in the presence of CAF were flat and extended, morphologically identical to cells in the presence of serum. In contrast, cells plated on to collagen in medium containing 200 $\mu\text{g ml}^{-1}$ BSA retained a rounded, refractile appearance (Fig. 1b). At concentrations of CAF which permitted half-maximal attachment of cells, a mixture of flat and rounded cells was apparent.

Attachment of cells to collagen in the presence of CAF was dependent on physiological concentrations of divalent ions, PyBHK cells failing to attach to collagen substrates in phosphate buffered saline (PBS) unless Ca^{2+} and Mg^{2+} were also present (Table 2).

When BHK cells, which contain high levels of CAF (ref. 9), were removed from confluent monolayers with trypsin-EDTA at levels of trypsin which completely remove surface CAF (ref. 9) their reattachment to collagen was also mediated by CAF with results paralleling those given for PyBHK (data not shown).

The demonstration¹⁶ that CAF purified from chick embryo fibroblasts agglutinates formalinised erythrocytes and that agglutination could be inhibited by substances containing free amino groups, prompted our assessment of their inhibitory properties during adhesion of cells to collagen. Inhibition was determined at two stages of CAF-mediated cell-adhesion: during CAF binding to collagen and during interaction of cells with CAF-collagen complexes. The results demonstrate that when 0.1 M L-lysine was present during the first but not the second incubation, cell attachment proceeded unimpeded, but with L-lysine present during only the second incubation, the flat, extended morphology of attached cells was completely inhibited, and the cells retained a rounded morphology. They did seem, however, to be more firmly associated with collagen than they were in the presence of E4-BSA alone, as judged microscopically by shaking the dish and observing that in E4-BSA the cells floated freely, whereas in the presence of serum plus inhibitor, the cells were not freely floating but loosely associated with the collagen. Quantification of the extent of this association was difficult because the cells were readily dislodged by gentle washing.

Table 3 Inhibition of serum- or CAF-mediated adhesion of PyBHK cells to collagen

First incubation	Second incubation	Morphology
10% Serum	Media	Flat, extended
CAF	Media	Flat, extended
10% Serum + 0.1 M L-lysine	Media	Flat, extended
CAF + 0.1 M L-lysine	Media	Flat, extended
10% Serum	Media + 0.1 M L-lysine	Rounded
CAF	Media + 0.1 M L-lysine	Rounded
10% Serum + 0.1 M D-galactose	Media	Flat, extended
CAF + 0.1 M D-galactose	Media	Flat, extended
10% Serum	Media + 0.1 M D-galactose	Flat, extended
CAF	Media + 0.1 M D-galactose	Flat, extended

Final concentration of CAF 100 $\mu\text{g ml}^{-1}$. The following inhibitors also prevented adhesion of cells to collagen when added during the second incubation: D-mannosamine (0.1 M), L-arginine (0.1 M), D-glucosamine (0.1 M) and D-galactosamine (0.1 M). The following compounds failed to inhibit cell adhesion when present at either incubation: D-glucose (0.1 M), glycine (0.1 M), and α -methyl-D-mannoside (0.1 M). Substances were tested for inhibition of cell attachment to collagen in the presence of 10% calf serum or purified CAF as follows: collagen-coated 30-mm dishes were incubated 1 h at 37 °C with 0.5 ml medium with or without 0.1 M concentration of the test substances. All inhibitors were dissolved in PBS and adjusted to pH 7.4 before use. Dishes were then washed twice with E4-BSA (20 °C) and 1 ml E4-BSA was added containing 5×10^5 PyBHK cells with or without the test substance. After an additional 1.5 h at 37 °C, dishes were inspected microscopically to ascertain cell morphology and adhesion.

Inhibition of adhesion by L-lysine could not be attributed to cytotoxicity. When cells were exposed to 0.1 M L-lysine, washed twice with E4-BSA and plated on to collagen pre-incubated with serum, the cells attached and flattened. Thus, cell viability was unaffected by the inhibitor.

As an additional control, D-galactose, which was negative in agglutinating erythrocytes¹⁶, was assayed for its ability to inhibit cell adhesion. It failed to inhibit cell attachment at either incubation step (Table 3).

Similar experiments employing CAF extracted from BHK cells (Table 3) showed that the pattern of inhibition with the purified protein was identical to that obtained in experiments with serum plus inhibitor.

It has been demonstrated¹⁷ that transformed cells which lack surface CAF are capable of synthesising this protein and secreting it into the medium. Therefore, conditioned medium from normal and transformed cells were assayed for active CAF. The results (Table 4) demonstrate that factor secreted from either BHK or PyBHK cells was capable of mediating the adhesion of cells to collagen. L-lysine, an inhibitor of serum and CAF-mediated attachment during the second incubation step, also prevented adhesion in the presence of conditioned medium.

It seems that CAF, extracted from normal cells by urea, is active in promoting attachment of cells to collagen. The attachment is dependent on dose, with 50% attachment occurring at 25 $\mu\text{g ml}^{-1}$ CAF (Table 1). Using the method of urea extraction with chick embryo fibroblasts, Yamada *et al.*¹⁶ have extracted CAF in greater than 95% purity. With BHK cells, however, purity was never greater than approximately 60% as judged by electrophoresis in polyacrylamide gel, so that 25 $\mu\text{g ml}^{-1}$ represents an overestimate of CAF added.

It has been demonstrated¹⁷ that CAF is secreted by SV40-transformed human fibroblast lines although these cells do not retain membrane-bound CAF. It is also known that CAF is turned over from the surface of normal fibroblasts⁹. We have demonstrated (Table 4) that CAF released into the medium from normal or transformed cells is active in promoting attachment of cells. Therefore, CAF produced by transformed cells is a biologically functional molecule and might be absent from the plasma membrane of transformed cells because of some other defect of the transformed cell plasma membrane.

CAF is a normal constituent of serum¹², and Klebe¹⁴ has shown that a serum factor necessary for the adhesion of cells to collagen has a molecular weight greater than 200,000, binds to DEAE, and requires physiological concentrations of Ca^{2+} and Mg^{2+} to express its function. CAF also has a molecular weight greater than 200,000 (refs 7, 8 and 12), binds to DEAE (unpublished observations) and requires Ca^{2+} and Mg^{2+} to promote adhesion (Table 2). In addition, we have found (Table 3) that whether the source of adhesion factor was serum or BHK extracts, the pattern of inhibition of adhesion by various substances was identical. Since in all these properties the serum factor corresponds to CAF it seems possible that they are identical. The activity of the serum factor was, however, resistant to 10 $\mu\text{g ml}^{-1}$ trypsin¹⁴, whereas CAF was susceptible to lower concentrations¹⁶. Therefore, additional work is needed to clarify the relationship between these factors.

Several types of transformed sarcoma-producing cell lines contain detectable levels of CAF (ref. 18) as determined by lactoperoxidase-catalysed iodination. Because PyBHK cells produce biologically active CAF (Table 4), it is likely that the CAF retained by some of the sarcoma-producing lines

Table 4 Promotion of adhesion of cells to collagen by conditioned medium (CM)

Source of conditioned medium	First incubation	Second incubation	Morphology
BHK	CM	Medium	Flat, extended
BHK	CM+0.1 M L-lysine	Medium	Flat, extended
BHK	CM	Medium+0.1 M L-lysine	Rounded
PyBHK	CM	Medium	Flat, extended
PyBHK	CM+0.1 M L-lysine	Medium	Flat, extended
PyBHK	CM	Medium+0.1 M L-lysine	Rounded

Confluent monolayers of BHK and PyBHK cells on 6-cm dishes were washed three times with E4, replenished with 2 ml serum-free E4 and incubated for 24 h. The conditioned medium was removed, spun at 2,000 r.p.m. for 2 min in siliconised test tubes to pellet contaminating cells, dialysed for 48 h against distilled water, lyophilised in siliconised flasks and dissolved in E4-BSA immediately before use. Assays for cell adhesion in conditioned medium were identical to those described previously.

That a minor component of the BHK-urea extract was not responsible for promotion of adhesion is supported by the fact that: urea extracts of PyBHK cells which lack CAF failed to promote attachment; CAF and adhesion activity are both non-dialysable; and inhibition of adhesion by specific compounds exactly parallels results obtained by Yamada *et al.*¹⁶ with pure CAF to inhibit the agglutination of erythrocytes. Production of an antibody specific for CFA, followed by inhibition experiments, would allow a direct demonstration of the role for CAF in cell attachment and spreading.

Specific inhibitors are effective in preventing firm cell adhesion and spreading when added during the second incubation (Table 3). As judged microscopically during gentle shaking of the dish, however, cells in the presence of CAF plus inhibitors were weakly adherent to the collagen when compared with cells plated solely in E4-BSA. In the presence of inhibitors, the cells retained a rounded morphology and failed to flatten and extend. It is unclear whether CAF is involved in both attachment and spreading, of which only the spreading is inhibited by the amino-containing substances; or whether another factor present in serum, urea extracts, and conditioned medium is responsible for attachment and CAF for cell spreading.

will also prove to be biologically active. Collagen is the natural substratum for fibroblasts *in vivo* and since CAF mediates the adhesion of these cells to collagen, it would be of great interest to correlate the presence or absence of CAF on transformed cells with tumour metastasis *in vivo*.

As mentioned, purified CAF can agglutinate formalinised sheep erythrocytes¹⁶. We believe the designation CAF to be broad enough to encompass the functions of both agglutination and adhesion.

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Temporary intestinal hypoxia induced by degradable microspheres

In all mammalian cells and tissues studied, the adequate presence of molecular oxygen during irradiation enhances the effects of ionising radiations of low linear energy transfer (LET), such as X rays, γ rays or electrons, by a factor of 2 or 3, compared with the effects seen after irradiation in poor oxygenation conditions^{1,2}. In radiotherapy, this oxygen enhancement ratio (OER) is assumed to be generally effective in all healthy tissues, whereas in poorly oxygenated tumour cells the partial radioprotection through hypoxia is believed to be efficient to an extent that the probability of tumour sterilisation may be affected critically^{3,4}. It has been suggested that the relative concentration of oxygen in healthy and tumour tissues could be changed to improve the relative probability of curing the disease and of reducing clinical complications caused by radiation damage of healthy tissues. To this end, the introduction of 'high LET' radiations for which the oxygen effect is of little importance, is presently being attempted⁵. Attempts are also being made to increase the oxygen tension in poorly oxygenated areas by treatment of patients with hyperbaric oxygen during irradiation⁶. In experiments in our laboratories, we have tried various means of changing the oxygen concentrations in tissues to be irradiated by chemical removal of oxygen⁷ or by mechanical obstruction of the blood flow (K.E.A., J.O.F., C. Clintenberg, B.L., B.R. and S.Ö., unpublished). We are now investigating, in experiments on pigs, the possibility of attaining temporary local hypoxia in intestinal segments by obstructing the blood flow at the level of the arterioles by intra-arterial injection of cross-linked starch microspheres, 43 μ m in diameter, that are degraded by the amylase activity of the serum⁸. With a proper choice of the degree of cross linking and of particle concentration, a hypoxic period of about 5 min duration, followed by rapid normalisation, was achieved.

Three female pigs of native breed (15-20 kg) were used under Nembutal anaesthesia. A high midline abdominal laparotomy was carried out and the base of the superior mesenteric artery was freed and catheterised using an Argyle No. 5 baby feeding tube. The catheter was fixed at the base of the mesenteric artery by a purse string suture and brought, for access, through the left abdominal wall and subcutaneously to the left groin, a loop being left in the abdominal cavity. The abdominal incision was closed and a loop of the small intestine was made available for inspection and measurement through a second incision through the right flank. Oxygen tension was measured with a modified Clark platinum electrode⁹ put in direct contact with the mucous membrane of the jejunum through a small incision in the intestinal wall.

Treatment, aimed at removal of oxygen from the intestinal segment under study, was carried out by injection of 6×10^6 (a) or 30×10^6 (b) microspheres suspended in 8 or

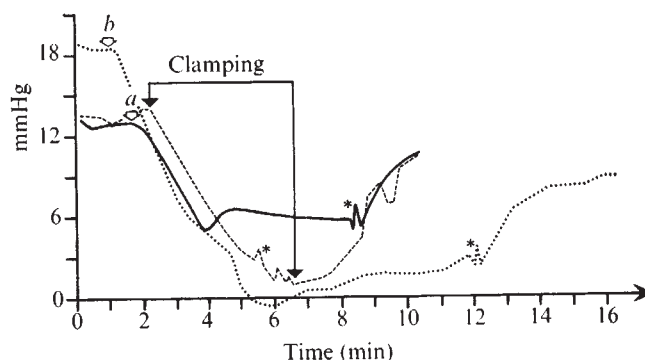


Fig. 1 Transient reduction in oxygen pressure on the jejunal mucous membrane after injection of 6×10^6 (a) or 30×10^6 (b) starch particles in the superior mesenteric artery of pigs. For comparison, the record seen after temporary mechanical 'clamping' of the vessel is also shown. *, Peristalsis.

40 ml Ringer's solution for periods of 6 to 15 s, respectively. Alternatively, for comparison, the blood flow in the superior mesenteric artery was obstructed using a mechanical clamp.

Typical results are shown in Fig. 1. Curves a and b represent the records of the oxygen pressure during the experiments. Qualitatively, the curves are similar, showing a rapid decrease in the oxygen pressure to low values that persist for a period of 4-7 min. At the end of this phase of hypoxia a sudden peristaltic activity was apparent, initiating the phase of restoration in which the oxygen pressure rapidly returned to normal within 2-4 min (this phenomenon is also reflected in the diagram). The greater number of particles was more efficient in reducing the oxygen pressure in the intestinal mucous membrane to near zero. With the exception that restoration took place more rapidly, clamping led to similar behaviour of the recorded curves. When the oxygen pressure dropped below about 6 mmHg the intestinal segment became clearly pale. The measured and visually observed changes were well correlated in time and quite reproducible.

The results show that the intra-arterial injection of degradable starch microspheres, as described, can be used to bring about temporary intestinal hypoxia. By proper choice of the amount and concentration of particles in the injected suspension, the period of hypoxia can be programmed so as to enable experimental radiotherapy to be carried out in hypoxic conditions, seemingly without risk of damage caused by ischaemia.

The technique suggested is now subject to detailed experimental investigation in combination with irradiation. Its application to clinical radiotherapy is also being considered.

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Metabolic adaptation to pregnancy shown by increased biosynthesis of insulin in islets of Langerhans isolated from pregnant rats

MANY reports have indicated that insulin secretion is increased in pregnant women^{1,2} although blood sugar levels may be unchanged, indicating an increased demand for insulin in human pregnancy. Similar findings have been reported for the effects of pregnancy on insulin secretion in pregnant rats^{3,4} which begin to respond with a significantly increased insulin secretion at a lower glucose level than those found in the normal female⁵. In addition, the secretory response is considerably greater in islets of Langerhans from pregnant than in normal rats, indicating an overall increased sensitisation of the islets from pregnant rats to the effects of glucose⁵. Glucose has a major role in the metabolism of the mammalian β cell, in that at physiological concentrations it is capable of stimulating both insulin secretion and biosynthesis^{6,7}. It is possible that increased insulin biosynthesis is necessary to support the increased secretory response to glucose in pregnancy, if the content of β cell insulin is to be maintained. We have therefore investigated whether the alterations in insulin secretion observed *in vivo* during pregnancy are paralleled by an enhanced insulin biosynthesis in isolated islets from pregnant rats, after exposure to increasing concentrations of glucose.

Islets of Langerhans were isolated by the method of Howell and Taylor⁸, from age-matched normal female and 19-d pregnant rats. Groups of 15–20 islets were incubated in 0.5 ml bicarbonate-buffered medium pH 7.4⁹, previously gassed with a mixture of 95% O₂ and 5% CO₂. The medium was supplemented with 1 mg ml⁻¹ bovine albumin and contained various concentrations of glucose, as indicated in Fig. 1, together with 20 μ Ci ml⁻¹ L-(4,5)-³H-leucine of initial specific radioactivity 40 Ci mmol⁻¹. The tubes were then incubated with gentle shaking for 3 h at 37 °C. After incubation, the islets were rinsed twice in phosphate-buffered 0.9% saline, pH 7.4 (PBS), containing 1 mg ml⁻¹ non-labelled L-leucine, and subsequently sonicated for 10 s in 150 μ l of PBS. After a tenfold dilution, samples of the sonicate were taken for determination of protein content¹⁰ using a crystalline bovine albumin standard. The synthesis of proinsulin and insulin in the pancreatic islets was estimated by determining the incorporation of labelled leucine into material reacting with insulin antibody (which also cross reacts with the precursor). The labelled insulin and proinsulin were separated from the rest of the islet proteins by incubation of a sample of the diluted islet sonicate with an excess of guinea pig anti-insulin serum (AIS) coupled to cyanogen bromide-activated Sepharose 4B (AIS-Sepharose)¹¹. Normal guinea pig serum (NGPS) coupled to activated Sepharose (NGPS-Sepharose) served as a control for nonspecific binding of labelled proteins to the Sepharose beads. The nonspecific adsorption of proteins to the NGPS-Sepharose was examined after treatment of the beads with 2 M acetic acid. It was found that a small peak of radioactivity was eluted with the void volume on Sephadex G-50 gel chromatography, but there was virtually nothing present in the insulin or proinsulin regions. Normally the radioactivity

bound to the AIS-Sepharose beads exceeded the nonspecific adsorption on NGPS-Sepharose by a factor of 2–5 in low (2 mM) and high (20 mM) glucose concentrations, respectively.

The biosynthetic response of the isolated islets to increasing glucose concentrations is shown in Fig. 1. Proinsulin-insulin biosynthesis was significantly greater ($P < 0.01$) in islets from pregnant compared with non-pregnant rats, at all the glucose concentrations studied. Green and Taylor⁵ have shown that there are increases in protein, DNA and insulin content of islets at day 19 of pregnancy, findings

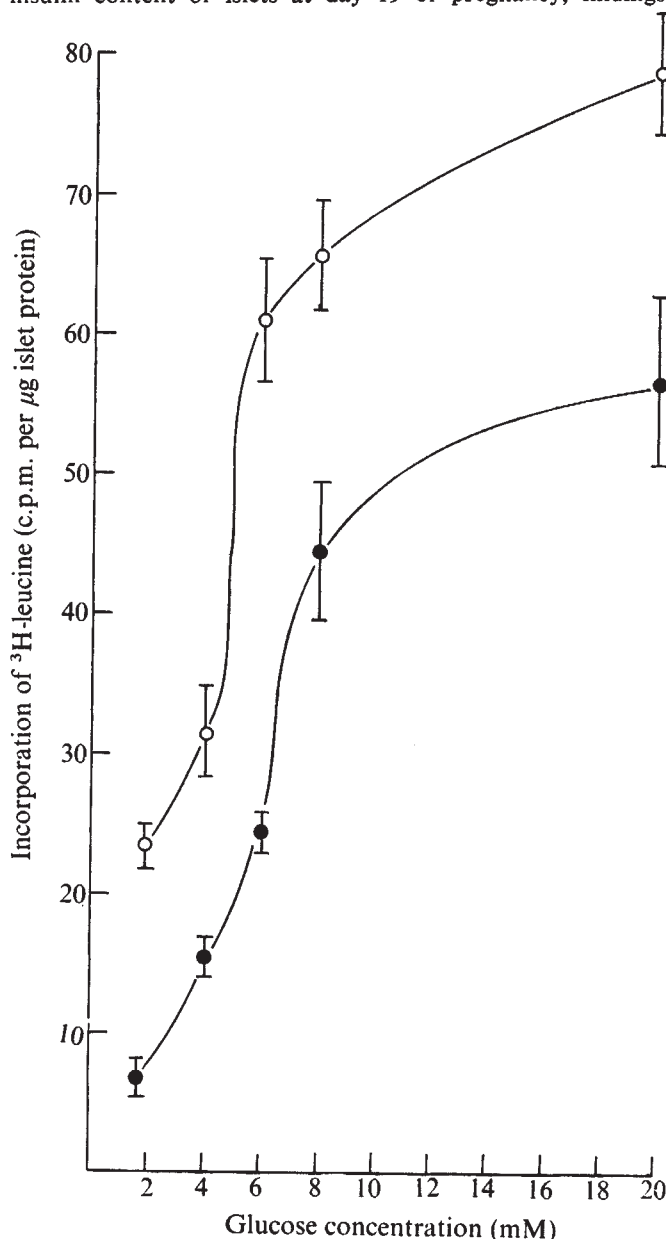


Fig. 1 Dose-response curve relating glucose concentration to the incorporation of L-4,5-³H-leucine into AIS-Sepharose-bound proinsulin-insulin in islets from normal (●) and 19-d pregnant rats (○). Each point represents a mean of at least eight observations, with $\pm$ s.e.m. shown as vertical bars. Groups of 15–20 islets were incubated, washed and sonicated as described in the text; 20- μ l samples of the diluted sonicates were then mixed with 1 ml PBS, supplemented with 2 mg ml⁻¹ bovine albumin, and containing either 20 mg AIS-Sepharose or the same weight of NGPS-Sepharose according to the method of Berne¹¹. The immune binding reaction was carried out in polythene tubes rotated end-over-end (20 r.p.m.) for 90 min at room temperature. The Sepharose beads were then washed six times with PBS and solubilised overnight in 0.8 ml Soluene 350 tissue solubiliser. Radioactivity was measured in a Beckman liquid scintillation counter after addition of 3 ml toluene-based scintillator.

confirmed by our islet protein estimations. Expressing incorporation per μg islet protein, however, we were able to nullify any differences in size and content of the isolated islets, thus enabling direct comparison of the biosynthetic response to glucose in islets from pregnant and non-pregnant rats. A sigmoidal curve is known to characterise the relationship between proinsulin–insulin biosynthesis and glucose concentration in normal islets¹². The threshold value for the stimulant action of glucose, however, is lower in islets from pregnant rats, and in addition the rate of change of insulin biosynthesis is considerably greater than in normal islets (Fig. 1). Similar findings have been reported in the rat for the effects of pregnancy on the insulin secretory response of the islets of Langerhans^{3–5}. On further examination of the sensitivity of the islets from pregnant rats to a glucose stimulus it is apparent that proinsulin–insulin biosynthesis has a lower threshold in terms of glucose stimulation than that for insulin release. Insulin biosynthesis is therefore more sensitive to glucose than is insulin release, and this will prevent the insulin stores of the β cells from becoming depleted at low glucose concentrations. This may be particularly important during pregnancy where blood sugar levels in fasted pregnant rats are significantly lower than those in fasted normal rats⁵, such depressed glucose levels perhaps being associated with the increased plasma insulin levels reported in fasted rats during pregnancy³.

The reasons for the heightened sensitivity of islets to glucose during pregnancy are at present uncertain. It has been suggested that there may be a possible involvement of increased adenylate cyclase activity or increased cyclic AMP concentrations in β cells, in controlling the altered secretory response¹³. Studies on the actions of theophylline and cyclic AMP at various glucose concentrations on biosynthesis of insulin compared with insulin secretion, prompted the suggestion by Schatz *et al.*¹⁴, that the adenylate cyclase system besides regulating insulin secretion is involved in insulin biosynthesis. Other workers have postulated an increased sensitivity of a membrane receptor as a possible reason for changes in the sensitisation of islets to the effect of glucose¹⁵, and changes of this type could also be operating during pregnancy. Whatever the mechanism by which it occurs, it is of great interest that at least three indices of islet activity are simultaneously increased in pregnancy. These are insulin release, insulin biosynthesis and islet glucose oxidation¹⁶.

Further work is now in progress to elucidate more exactly the biochemical mechanisms by which the islets of Langerhans adapt to pregnancy.

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Electrophysiological evidence for coupling between β cells of pancreatic islets

It has been shown that the β cells of pancreatic islets exhibit electrical activity when stimulated with insulin releasing agents^{1–3}. There is evidence that this activity is directly correlated with the insulin release and that it has an important role in the secretory process^{3,4,5}. More recently it was documented that β cells within an islet are connected by gap junctions^{6,7} which are considered as low resistance pathways (ref. 8 and refs therein). The question therefore arises whether the electrical activity can spread electrotonically from one cell to another within an islet. To further elucidate this question the electrical activity from two different cells was measured by simultaneous impalement with two microelectrodes.

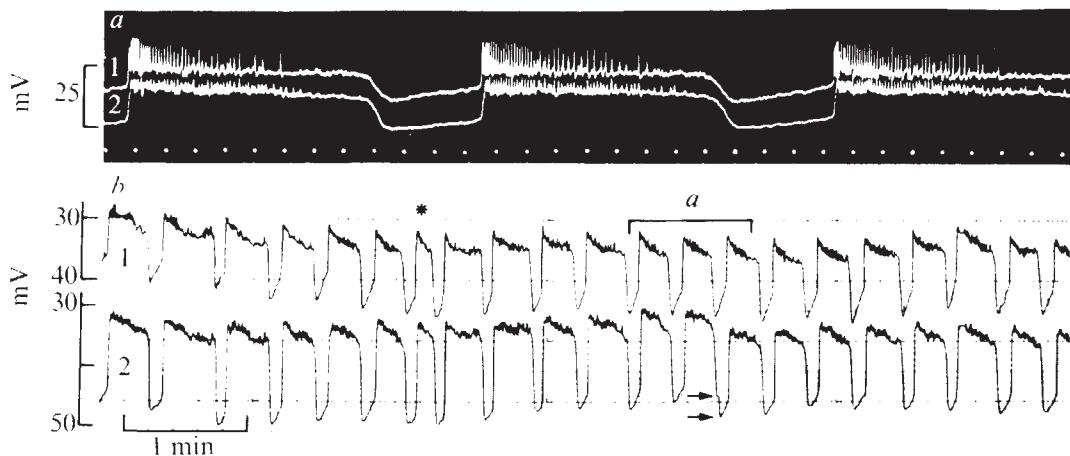
The experiments were done on microdissected islets of the mouse pancreas as described previously². The islets were continuously perfused in a small Plexiglas chamber (volume ~1 ml) with a Krebs–Henseleit solution gassed with 95% O₂ and 5% CO₂. The temperature was kept constant at 37 °C. The basal medium had the following composition (mM): glucose 2.8, NaCl 103, KCl 4.7, CaCl₂ 2.56, MgCl₂ 1.13, NaHCO₃ 25, NaH₂PO₄ 1.15, sodium pyruvate 4.9, sodium fumarate 2.7, sodium glutamate 4.9. When the glucose concentration was increased to 11.1 or 16.6 mM, NaCl was reduced by an appropriate amount to maintain the osmolarity.

The membrane potential was measured with microelectrodes filled with 2 M potassium citrate. Because the β cells are small (diameter ~10 μm) simultaneous recording from two cells was difficult and high resistance electrodes (tip resistance >200 M Ω) were necessary to obtain stable and long lasting impalements. The electrodes were mounted on two independent micromanipulators and impaled at an angle of approximately 10°. The distance between their tips was about 50 μm before impalement. Since the β cells in mouse islets are surrounded by a layer of α cells⁹ they are invisible. Therefore, they could only be identified by their characteristic electrical activity in high glucose concentrations. Membrane potentials were recorded on a double beam oscilloscope (Tektronix 565) and a double trace chart writer (W+W 1200).

The glucose-induced electrical activity is characterised by a regular burst pattern^{2,5}. It consists of periodic oscillations of the membrane potential (slow waves) between a depolarised state with superimposed fast spike activity, and a polarised, silent state (Fig. 1). The burst activity appears only in glucose concentrations which also stimulate insulin release². The duration of the slow waves is strongly glucose dependent; it increases as the glucose concentration is raised^{2,10}. The amplitude and the duration of the superimposed spikes are unaffected by a change of the glucose concentration.

When the electrical activity was simultaneously recorded from two cells within the same islet the slow waves were highly synchronised in 6 out of 7 double impalements; only in one case were the slow waves asynchronous. The synchronisation is demonstrated in Fig. 1. It is obvious that there was a strict temporal relationship between the depolarising as well as the repolarising phases of the slow waves in the two records. This relationship was unaltered also when during the regular burst pattern a slow wave with a shorter duration was interposed (Fig. 1b). The

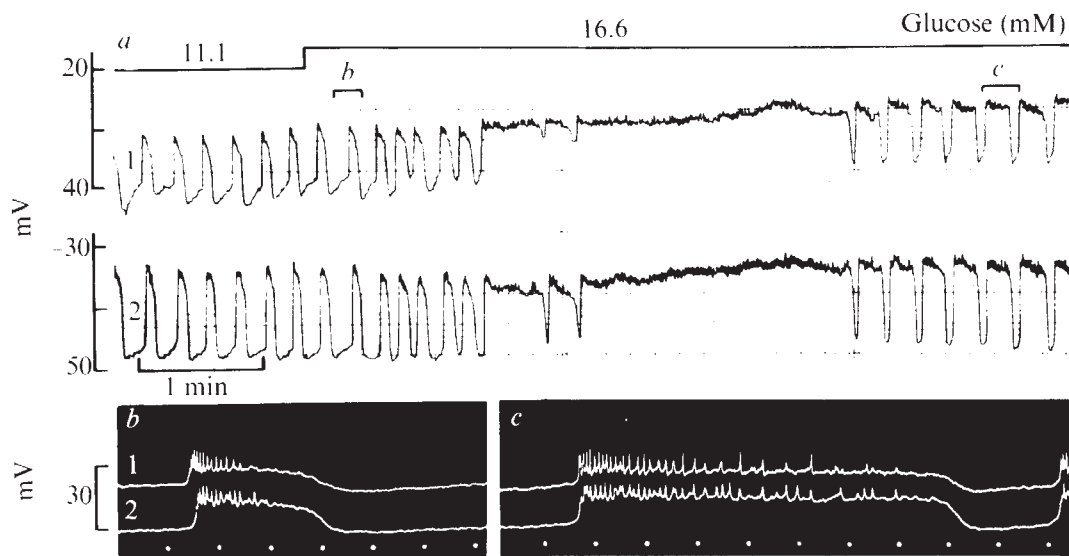
Fig. 1 Simultaneously recorded burst activity of two β cells perfused with 16.6 mM glucose. *a*, Oscilloscope record of the three bursts marked by the bar in record *b*. Distance between two subsequent points corresponds to 2 s. *b*, Train of bursts recorded with a chart writer. Trace 1 and 2 in *a* correspond to trace 1 and 2 in *b*, respectively. At the two arrows in *b* the offset was changed by about 3 mV. The asterisk in *b* denotes bursts with a short duration interspersed between longer bursts. Note that the full amplitude of the spikes is not recorded in the chart recordings because of the inertia of the recording system.



differences in the membrane potential values of the two cells shown in Fig. 1 are likely to be due to differences of the recording conditions and not to different properties of the impaled cells. As shown in Fig. 2, the synchronisation was also maintained when the external glucose concentration was changed, although the pattern of activity was markedly altered. It is unlikely that the synchronised

The results show that the electrical activity is synchronised in a high percentage of β cells which are located closely together within an islet. This synchronisation concerns mainly the slow waves whereas the fast spike activity seems to be synchronised only partially. It has been established that the β cells are connected by gap junctions^{6,7}; therefore it is probable that the synchronisation is due to

Fig. 2 Effect of changing the glucose concentration on the simultaneously recorded burst activity of two β cells. *a*, Continuous ink recording showing the time course of burst activity before and after changing the glucose concentration from 11.1 to 16.6 mM. *b* and *c*, Oscilloscope records taken at the times marked by bars in *a*. Time interval between two subsequent points in *b* corresponds to 2 s.



activity was recorded from the same cell because the micro-electrode tips were separated by distances which were several times the diameter of the β cell. This conclusion is supported by the observations shown in the oscilloscope records of Figs 1*a*, 2*b* and *c*; these records reveal that in contrast to the slow waves the superimposed spike activity was not identical at the two recording sites. From Fig. 3, which shows the spike activity on a faster time base, it becomes obvious that the spikes were well synchronised during the initial part of the burst (left half of Fig. 3) while later on, when the frequency decreased, the synchronisation was less pronounced. Thus, the spikes in the upper record of Fig. 3 appeared with an increasing delay compared with those in the lower trace (see spikes 20 to 30). Moreover, single spikes in one record were sometimes not accompanied by a corresponding spike in the other record (for example spike 30). Furthermore, if corresponding spikes were present in the two records the ratio of their amplitudes varied considerably; for instance spike 22 was larger in the lower than in the upper record, whereas for spike 29 the reverse was true.

electrical coupling. Although the ionic mechanisms producing the electrical activity in β cells so far are poorly understood it has been suggested that particularly the glucose-induced slow waves are an important link in triggering and regulating insulin release in the individual cell^{2,5,10,11}.

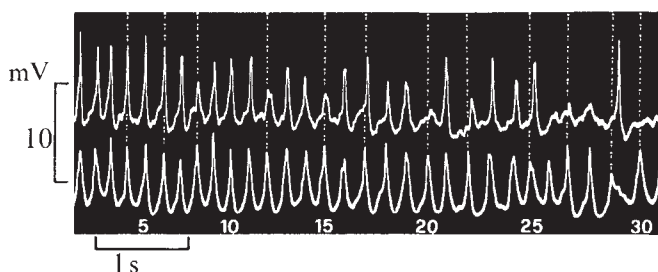


Fig. 3 Simultaneous oscilloscope records of spike activity of two β cells during the plateau phase of a slow wave. To allow comparison of the temporal relationship between spikes in the two records, the spikes have been numbered and dotted lines have been traced. Glucose concentration 16.6 mM.

Since many of these cells seem to be coupled electrically, it is possible that the slow wave originates in one cell and stimulates the coupled cells by electrotonic spread. From this point of view it is conceivable that within an islet the glucose molecule stimulates only a limited number of 'pacemaker cells' which then induce electrically the burst activity and insulin release in the coupled cells. The 'pacemaker cells' may be located in close vicinity to the blood capillaries and, therefore, be the first to come into contact with the glucose.

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Decrease of Na^+ conductance during desensitisation of the frog endplate

THE membrane depolarisation of the frog endplate caused by acetylcholine (ACh), either released from the nerve terminals or applied externally, decreases when such applications of ACh are persisted or repeated^{1,2}. This phenomenon, known as 'desensitisation' or 'inactivation' of the receptor to ACh, was first analysed by Katz and Thesleff³ and was suggested to be caused by a change in the receptor molecule from an effective to a refractory state. Subsequent investigations revealed that the rate of this desensitisation depends both on the extracellular concentration of Ca^{2+} ($[\text{Ca}^{2+}]_0$)^{3–5} and on membrane potential⁶; being speeded up by increasing $[\text{Ca}^{2+}]_0$ or hyperpolarisation of the membrane. These observations and others, demonstrating that the endplate membrane became permeable to Ca^{2+} during the action of ACh (ref. 7), led to the hypothesis that desensitisation was brought about by transmembrane movement of Ca^{2+} and the combination of the ions with anionic sites on the inner surface of the endplate membrane^{4,5}. The question then arises of whether desensitisation is caused by molecular changes in the ACh receptor or by those of the subsequent step directly controlling the ionic conductance of the endplate membrane. This subsequent step may involve a macromolecule which has been termed "ion channel" or "ion conductance modulator"⁸. In an attempt to answer this question, we measured changes in the equilibrium potential of the endplate membrane during desensitisation, using a voltage-clamp technique^{9,10}.

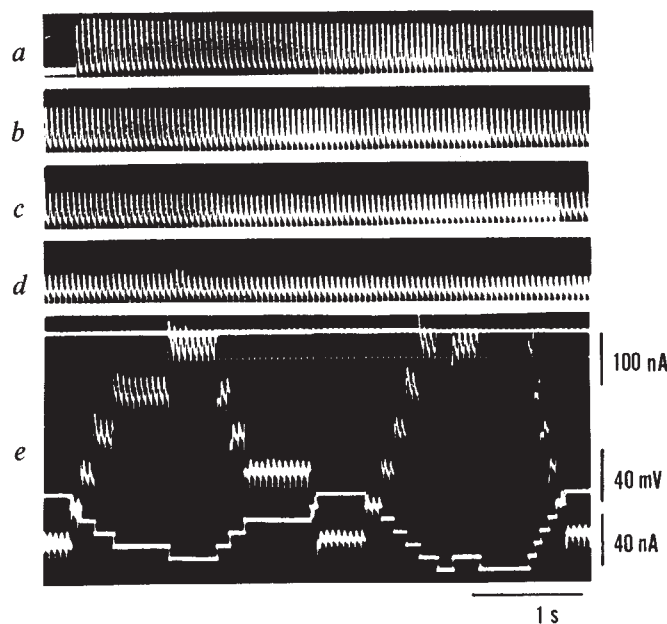
Sciatic nerve–sartorius muscle preparations were isolated from frogs (*Rana nigromaculata*). Conventional intracellular recording techniques and a voltage clamp circuit were as used previously¹¹. Ionic composition of Ringer's solution was as follows (mM): NaCl, 115; KCl, 2.0; CaCl_2 , 1.8; NaH_2PO_4 , 0.7; Na_2HPO_4 , 1.3. Experiments were carried out at room temperature (20–22 °C). Electrodes for iontophoresis of ACh (2 M) had tip resistances of 100–140 M Ω and a braking current of a few nA was applied constantly.

Endplate currents (ACh-induced current) were evoked by iontophoretic applications of ACh at a rate of 0.2 Hz and at different levels of membrane potential. Thus, the endplate conductance (G_{epc}) and its equilibrium potential (E_{epc}) before desensitisation occurred were obtained (Fig. 2a). The endplate membrane was then desensitised by generating a series of ACh-induced currents at a relatively high rate (20 Hz), as

demonstrated by Magazanik and Vyskocil¹² and as shown in Fig. 1. During repeated applications, the amplitude of ACh-induced currents decreased and after 20 s became 25% of the initial level. When the desensitisation had developed sufficiently, the level of membrane potential was changed from the holding potential of -50 mV up to -105 mV. Accordingly (Fig. 2a) changes in G_{epc} and E_{epc} during desensitisation can be compared with those before desensitisation occurred. There was a marked shift of the E_{epc} to a more negative value (the mean shift; -25.4 ± 5.6 mV (s.e.), $n = 9$) during desensitisation, accompanied by a decrease in the G_{epc} ($37.3 \pm 2.9\%$ of the control value, $n = 9$). It is unlikely that the lines, shown in Fig. 2a, relating the amplitude of the ACh-induced current to membrane potential before and during desensitisation will converge to the same membrane potential. In fact, in some cells, there was complete disappearance of ACh-induced currents at -50 mV during desensitisation, whereas the E_{epc} obtained by extrapolation before the development of desensitisation was -10 mV. These results indicate that a decrease in the Na^+ conductance (G_{Na}) of the endplate membrane would occur in these conditions.

Before this conclusion is accepted, however, the possibility should be considered that the shift in E_{epc} during desensitisation might be caused by a change in the equilibrium potential for Na^+ (E_{Na}) because of an influx of Na^+ during repeated activation of the endplate membrane. To test this possibility, the E_{epc} was measured during repetitive generation of ACh-induced currents in low $[\text{Ca}^{2+}]_0$, where the rate of desensitisation was very slow. In this situation, if the shift of the E_{epc} during repetitive applications of ACh is a result of a change in the E_{Na} , there would be a similar, or even a greater change, in the E_{epc} than that at normal $[\text{Ca}^{2+}]_0$, since the decrease in the amplitude of ACh-induced currents during repeated activations is smaller at low $[\text{Ca}^{2+}]_0$ (Fig. 2b). Even after the generation of 400 ACh-induced currents, there was only a small change in E_{epc} during applications of ACh at 0.45 mM $[\text{Ca}^{2+}]_0$, eliminating the possibility that the negative shift of E_{epc} during desensitisation seen at 1.8 mM $[\text{Ca}^{2+}]_0$ was the result of accumulation of Na^+ in the intracellular space. The change in the E_{epc} therefore seems to be caused by the reduction of G_{Na} .

Fig. 1 ACh-induced currents evoked by repetitive applications of iontophoretic pulses (1 ms, 50 nA) at a rate of 20 Hz. Membrane potential was changed after ACh application for about 20 s, when sufficient desensitisation had occurred. a–d, ACh-induced currents; e, upper, middle (seen at the right- or left-hand margins) and lower (seen at the right- or left-hand margins) traces are the current for iontophoresis of ACh, membrane potential and ACh-induced current, respectively.



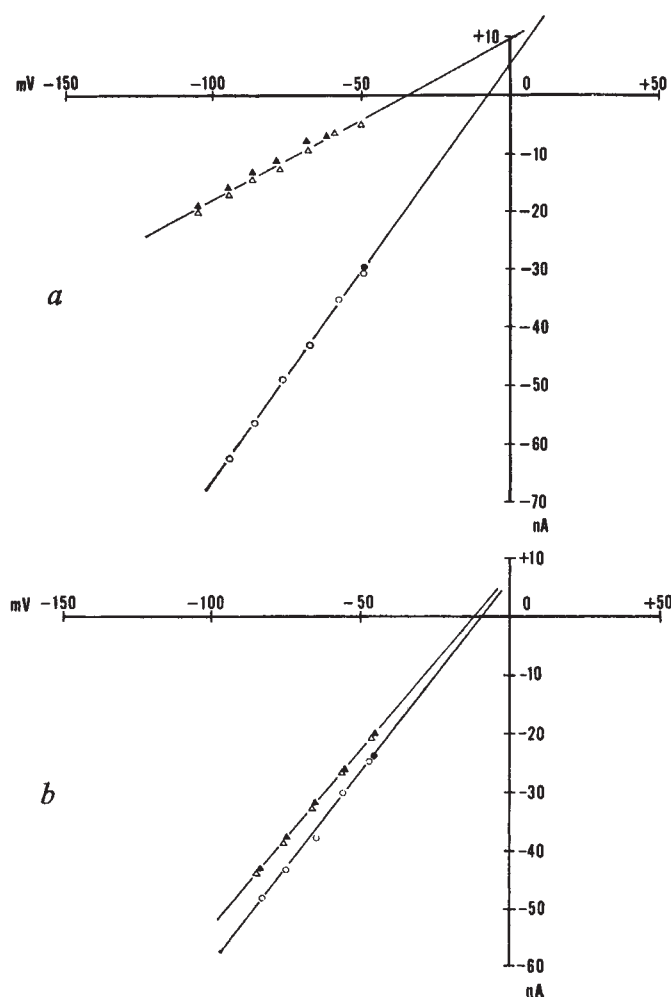


Fig. 2 Relationship between the peak amplitude of ACh-induced currents (ordinates) and membrane potential (abscissae) measured before and during desensitisation at 1.8 mM $[Ca^{2+}]_0$ (a) and 0.45 mM $[Ca^{2+}]_0$ (b). $\circ$, $\bullet$, Amplitude of ACh-induced currents generated at 0.2 Hz. Δ , $\blacktriangle$, Amplitude of the currents recorded after generation of about 400 currents at 20 Hz. Recorded by shifting the membrane potential in hyperpolarising directions ($\circ$, Δ); depolarising directions ($\bullet$, $\blacktriangle$). Membrane potential was held at -50 mV in both a and b to decrease the rate of desensitisation so that the equilibrium potential during desensitisation could be measured reliably.

of the endplate membrane. This finding points directly to the idea that the site responsible for desensitisation is the ion channel directly controlling the conductance of the endplate membrane⁶.

We also studied effects of high $[Ca^{2+}]_0$ on the ACh-induced currents which were evoked at a low rate (0.2 Hz) of ACh application, when there was no observable desensitisation. When $[Ca^{2+}]_0$ was increased from 1.8 mM to 7.2 mM, the amplitude of ACh-induced currents gradually decreased and reached a steady level. In these circumstances, there was a decrease in G_{epc} ($71 \pm 6\%$, $n = 7$) and a negative shift of E_{epc} (-10.3 ± 1.0 mV, $n = 7$), indicating a decrease in G_{Na} . These observations are in agreement with the original findings of Takeuchi⁷. In view of similar decreases in the G_{Na} , we suggest that two different effects of high $[Ca^{2+}]_0$ —namely an increase in the rate of desensitisation and a decrease in the endplate conductance—are caused by a common mechanism, which may be explained as follows. It was proposed^{4,5}, that a major factor controlling the speed of desensitisation is a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) produced by activation of Ca^{2+} conductance. If this is the case, our present experimental findings indicate that an increase in $[Ca^{2+}]_i$ eventually decreases G_{Na} of the endplate membrane. When

$[Ca^{2+}]_0$ is increased, $[Ca^{2+}]_i$ is also expected to rise, even at a low rate of ACh application. This leads to a similar decrease in the G_{Na} . It may thus be possible that desensitisation is caused by the acceleration of the mechanism which causes a decrease in G_{Na} of the endplate membrane by intracellular Ca^{2+} .

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Elevated levels of enkephalin in morphine-dependent rats

NUMEROUS theories propose mechanisms regulating opiate dependence. Hughes^{1,2} elegantly identified a morphine-like factor “enkephalin” which mimics effects of morphine on nerve plexus in smooth muscle in a fashion that can be antagonised by opiate antagonists, while a compound with the same properties has been identified in brain extracts by competition for opiate receptor binding^{3–6}. Enkephalin activity has a regional and subcellular distribution similar to that of the opiate receptor^{7,8} and was first shown to be a mixture of two peptides H-Tyr-Gly-Gly-Phe-Met-OH and H-Tyr-Gly-Gly-Phe-Leu-OH by Hughes *et al.*⁹ with a preponderance of the methionine peptide in pig brain⁹. Independently, we identified the same two peptides in bovine brain but with four times more of the leucine than of the methionine peptide^{10,11}. Brain enkephalin differs from substances in pituitary gland and hypothalamic extracts which mimic morphine^{12,13}.

Kosterlitz and Hughes proposed that reduced enkephalin release might explain tolerance and physical dependence to opiates¹⁴. Here we report enhanced enkephalin levels in brains of morphine-dependent rats, supporting the view that changes in enkephalin release account for symptoms of tolerance and physical dependence.

Enkephalin was extracted^{4,6} from brains of Sprague-Dawley rats (150–175 g weight) by homogenising the brain in ten volumes of 0.32 M sucrose in a glass tube with a Teflon homogeniser. The homogenate was centrifuged at 100,000g for 1 h, the pellet resuspended in two volumes of 10 mM Tris-HCl buffer (pH 7.7 at 25 °C) and incubated in a boiling water bath for 15 min, centrifuged at 100,000g for 1 h and the clear supernatant lyophilised. To separate endogenous enkephalin from the administered opiates, all the lyophilised brain extracts were purified on Biogel-P₂ columns. Samples were suspended in 0.1 M ammonium-acetate buffer (pH 7.4) and run through Biogel P₂ columns (26 × 1.1 cm, 200–400 mesh) prepared in the same buffer. Fractions of 0.6 ml were collected, lyophilised, suspended in 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) and assayed in triplicate in the opiate binding assay^{4–6}. When ³H-morphine was mixed with enkephalin, less than 1% of added ³H-morphine emerged in the enkephalin containing fractions. The opiate binding assay was performed in 200 μ l of 0.05 M Tris-HCl buffer containing membranes from Sprague-Dawley rat brains and 0.12 pmol ³H-naloxone (5 mCi/0.04 mg). Specific opiate binding is defined as the difference

Table 1 Enkephalin activity and opiate content in morphine-dependent, withdrawn and acutely injected rats

	Enkephalin activity (Units per g wet weight)	Opiate content (ng morphine equivalents per g wet weight)
Control	3.38±0.32 (8)	
Acute morphine†, 30 min	3.42±0.43 (8)	87.5±16.2
Acute morphine, 1 h	4.20±0.51 (8)	154.4±24.0
Acute morphine, 2 h	4.05±0.37 (8)	65.4±13.3
Acute morphine, 4 h	3.78±0.40 (6)	18.3± 5.4
One day morphine pellet‡	3.65±0.44 (5)	27.7± 3.6
Five day morphine pellet§	5.96±0.72* (10)	64.1± 7.1
Five day morphine pellet plus naloxone injection (1.0 mg kg ⁻¹)	3.26±0.51 (8)	96.5±14.1
Naloxone injection (1.0 mg kg ⁻¹)¶	2.84±0.47 (5)	31.0± 4.0

For determination of enkephalin activity and opiate content, rats were decapitated and brains minus cerebella were homogenised as described in the text. Enkephalin activity was separated from opiates (morphine or naloxone) by Biogel-P₂ chromatography (see text and Fig. 1) and assayed in an opiate binding assay^{4,6}. Each treatment was performed in 5–10 rats (indicated in parentheses) and enkephalin activity content quantified as described in the text. Data are means ± s.e.m. with the number of rats in parentheses. For methionine and leucine-enkephalins, 1 unit represents 1.25 pmol and 6.0 pmol respectively^{10,11}. Thus control rat brain would contain 4.2 pmol g⁻¹ or 20.3 pmol g⁻¹ levels of enkephalin if it were exclusively methionine or leucine-enkephalin respectively.

All samples were processed by Biogel-P₂ chromatography with assay of 50 fractions for each sample. There was no overlap between enkephalin values of 5-d pelleted rats and control rats or 5-d pelleted animals treated with naloxone.

*Significantly higher than values for control rats and morphine-dependent rats treated with naloxone, $P < 0.005$.

†Morphine sulphate (15 mg kg⁻¹) was injected intraperitoneally and animals decapitated for enkephalin extraction 30 min–4 h after injection.

‡Morphine pellets (75 mg) were inoculated subcutaneously 24 h before enkephalin extraction.

§One morphine pellet (75 mg) was inoculated. After 2 d two more morphine pellets (75 mg each) were inoculated together. Rats were decapitated and their brains extracted 5 d after inoculation of the first pellet.

||Rats were treated as in § except that naloxone (1.0 mg kg⁻¹ intraperitoneally) was injected 1 h before decapitation and brain extraction.

¶Naive animals were injected intraperitoneally with naloxone (1.0 mg kg⁻¹ intraperitoneally) and the brains extracted 1 h later as in ||.

in binding in the presence of 1 μM levallorphan from that in its absence. Enkephalin activity was quantified using a standard curve elaborated with different enkephalin concentrations. One unit of enkephalin is defined as that amount of enkephalin which yields 50% occupancy, determined according to Colquhoun¹⁵ assuming classical binding interactions. The amount of opiate in the brain extracts that was run through Biogel-P₂ columns was also determined in the opiate binding assay and quantified by comparison with morphine. Synthetic methionine- and leucine-enkephalins emerged from these columns in the same fractions as enkephalin extracted from rat brain^{10,11}. Chromatography on AG-1 columns also showed the same elution pattern for synthetic enkephalins and enkephalin activity extracted from rat brain. Recovery of added methionine- and leucine-enkephalins (80–90%) was the same for control and morphine pelleted rats.

One hour after a single injection of morphine (15 mg kg⁻¹ intraperitoneally) enkephalin levels increase about 25%, which attains statistical significance in some experiments (Table 1). No augmentation is detected 30 min after morphine injection and the levels of enkephalin then decline.

To evaluate the influence of tolerance and physical dependence, rats were implanted with slow-release morphine pellets and their brains assayed for enkephalin and morphine content 1 and 5 d later (Table 1; Fig. 1). At 1 d, enkephalin levels are no different from those of control

animals, though brain levels of morphine at this time are only 15% of those obtained 1 h after an acute injection of morphine, consistent with the reported slow release of morphine from such pellets in rats^{16,17}. Five days after initial pellet implantation and 3 d after the second pellets, when tolerance and physical dependence are maximal^{16,17}, enkephalin levels are increased 75% over control values; 1 h after precipitating abstinence in animals by naloxone treatment (1.0 mg kg⁻¹ intraperitoneally), enkephalin levels have returned again to control values. In naive rats the same dose of naloxone produces no alteration in enkephalin levels.

Changes in enkephalin activity are not simply related to presence in the brain of the opiate administered to the animals, because naloxone injection reduces enkephalin levels in 5-d morphine pelleted rats, while it increases the opiate content of the brain (Fig. 1; Table 1). Moreover, after a single injection of morphine, enkephalin levels are substantially lower than after 5 d of morphine pellets, yet with the acute injection of morphine, the total opiate content of the brain is two- to threefold higher than after pellet implantation.

Do the elevated levels of enkephalin in morphine pelleted rats represent the same substance as that detected in control animals? One unique characteristic of enkephalin activity is its enhancement by manganese but not by calcium ions and its depression by sodium but not potassium ions^{4,6}. As previously reported for crude and purified enkephalin preparations, in control rats as well as in morphine pelleted animals and rats treated with both morphine pellets and naloxone, ionic influences on enkephalin activity are essentially the same. Thus in all three cases, 15 mM and 100 mM sodium reduce enkephalin activity about 60 and

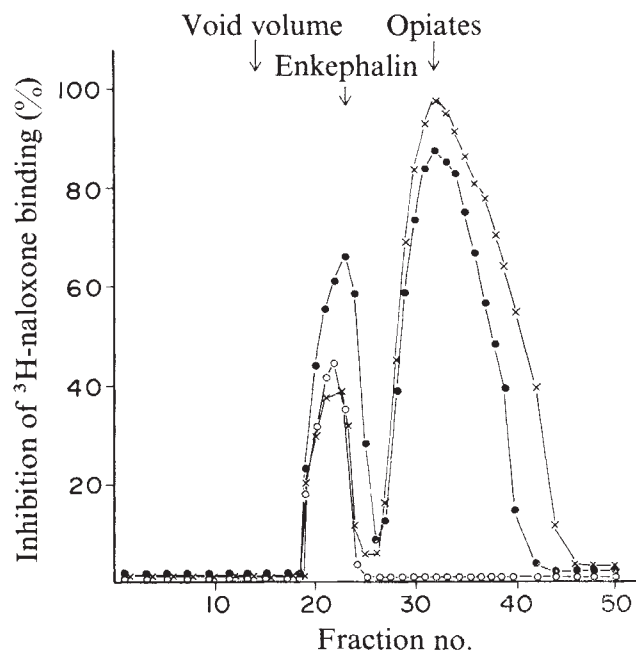


Fig. 1 Biogel-P₂ chromatography of enkephalin activity from control, morphine-dependent and morphine-dependent, naloxone-injected rats. Morphine dependence was elicited by implanting one morphine pellet (75 mg) for 2 d and then two other pellets (75 mg each) together for another 3 d before brain extraction. Abstinence was precipitated by intraperitoneal injection of 1.0 mg kg⁻¹ naloxone to rats 5 d after morphine pellet implantation, and the brains were extracted 1 h after naloxone injection. Each treatment was performed with 5–8 animals. Enkephalin was extracted, run through Biogel-P₂ column and assayed in the opiate receptor binding assay as described in the text. The same Biogel-P₂ assay was calibrated with ³H-morphine, synthetic methionine-enkephalin and non-radioactive insulin (molecular weight, 6,000). The negligible overlap of enkephalin and opiate profiles and their comparable magnitude ensures that enkephalin activity determinations are not affected to a great extent by opiate content of brain. ○, control; ●, morphine dependent; ×, morphine dependent + naloxone.

70% respectively, while the same concentrations of potassium have no effect. By contrast, 1 mM manganese increases enkephalin activity about 50–70% in all three groups of animals, although the same concentration of calcium has no appreciable influence. Elevated enkephalin levels in morphine-dependent rats remain apparent regardless of ionic manipulations.

How might these changes in enkephalin activity relate to opiate dependence? The jumping, wet shakes and diarrhoea associated with morphine abstinence are observable in our animals 5 min after naloxone injection, peak at about 15 min and subside by 60 min, as reported by others¹⁶. Coincident with the reduction in abstinence symptoms 1 h after naloxone administration, sensitivity to the analgesic effects of morphine returns almost to control levels¹⁷, when enkephalin levels have also declined to control values. Thus the changes in enkephalin activity after chronic treatment with morphine and induction of abstinence correlate with behavioural changes associated with tolerance and physical dependence.

Brain levels of dopamine and acetylcholine reflect changes in the firing rate of cholinergic and dopaminergic neurones^{18,19}. Cessation of firing is accompanied by an elevation of the levels of these transmitters. If enkephalin is a neurotransmitter or neuromodulator of analogous neuronal systems, elevations in enkephalin activity might reflect a slowing in the firing rate of "enkephalinergic" neurones. Conceivably large doses of morphine substitute for the physiological effects of enkephalin and, by a "feedback" message, cause the enkephalinergic neurones to reduce their activity as proposed by Kosterlitz and Hughes¹⁴. Such a mechanism could account for the behavioural manifestations of opiate tolerance and physical dependence. With acute morphine administration, opiate receptors are exposed both to morphine and enkephalin. With chronic morphine administration, enkephalin release slows or ceases, and opiate receptors, now exposed only to morphine, appear "tolerant". On abrupt cessation of opiate administration or with antagonist treatment, receptors are deprived both of the opiate and of enkephalin, resulting in "abstinence". As the firing rate of enkephalin neurones returns to normal, elevated levels of enkephalin decline to control values, and the organism is again normally responsive to opiates. Possibly, the "feedback" message to the enkephalin neurones is mediated by changes in cyclic nucleotides in postsynaptic cells containing opiate receptors²⁰.

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Prolactin suppresses release of luteinising hormone during lactation in the monkey

STUDIES with rats have shown that during lactation there is an inhibition of luteinising hormone (LH)-dependent physiological events, such as implantation¹, and a return to oestrus cyclicity². This inhibition has been shown to occur only during the intense suckling phase and it has been correlated with the high levels of prolactin present in the circulation at this time. Although exogenous prolactin could substitute for the effects of intense suckling, it could do so only under the permissive influence of minimal suckling stimulus. We have shown that there is, in these conditions, a lowering of LH levels, and that this is due to interference by prolactin with the pituitary responsiveness to LH-releasing hormone (LHRH) (K. Muralidhar, R. M. and N. R. M., unpublished). Using the lactating monkey, we have now demonstrated a similar inhibitory effect of prolactin on pituitary responsiveness to LHRH, suggesting a mechanism by which amenorrhoeic conditions are maintained during lactation.

Four female bonnet monkeys (*Macaca radiata*), in the second month of lactation, were caged individually with their babies and maintained in standard laboratory conditions³. Synthetic LHRH (100 µg AY-24, 031-4, given by Dr F. Labrie) in 1 ml 0.9% NaCl was administered through the femoral vein. LHRH was first injected while the mother was suckling her baby and blood was withdrawn by venepuncture 10, 20 and 40 min later. The baby was then separated from the mother for 24 h, at the end of which period a second injection of 100 µg LHRH was given, followed by blood sampling at the intervals indicated above. After a rest of 2–3 weeks the same monkeys were used for the prolactin inhibition experiment. The experimental protocol was identical to that described above, except for the intramuscular injection of 5 mg ovine prolactin (NIH-P-S5)

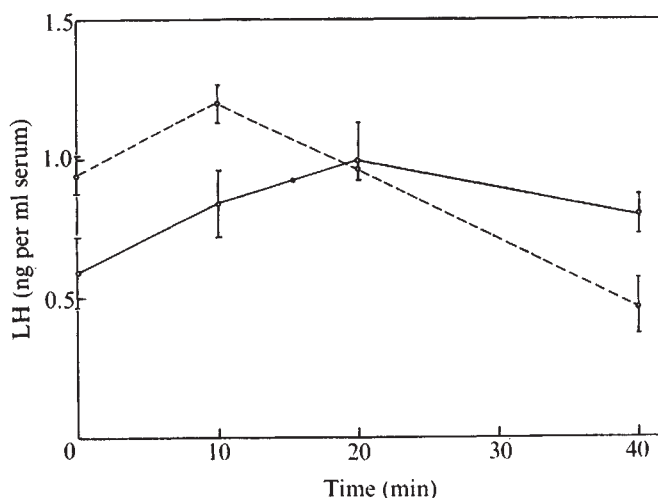


Fig. 1 Effect of suckling on pituitary response to LHRH. Four monkeys in the second month of lactation were used. LH was measured in duplicate in 0.1-ml samples by the double-antibody radioimmunoassay, using human LH (given by Dr C. H. Li), for iodination and standard and human chorionic gonadotrophin- β antiserum (NIAMDD, NIH) as a source of antibody. This assay accurately measured LH in the range of 0.1–5 ng. —, Suckling; ---, non-suckling.

in 0.5 ml 0.9% NaCl 1 h before administration of LHRH. In all experiments a control blood sample was taken 30 min before injection of either prolactin or LHRH. Serum LH was estimated by the double-antibody radioimmunoassay at 37 °C as described previously⁴⁻⁶ (details in Fig. 1).

Administration of LHRH resulted in an increase in serum of LH within 10 min, in both the suckling group and those from which the suckling stimulus was withdrawn for 24 h (Fig. 1). Whereas this increase in LH levels was maintained until 40 min in the suckling group ($P < 0.02$ between control and 40-min sample), LH reached control values in the non-suckling group by 20 min ($P > 0.5$ between control and 20-min sample). The basal level of LH was significantly increased after withdrawal of the suckling stimulus for 24 h (from 0.6 ± 0.12 ng ml⁻¹ to 0.95 ± 0.07 ng ml⁻¹, $P < 0.001$).

Administration of 5 mg prolactin 1 h before injection of LHRH resulted in a suppression of LH release into the circulation only in the group in which the suckling stimulus was present (Fig. 2a). This suppression of LH continued until 40 min after injection of LHRH. At any given time interval tested, LH levels in the prolactin-treated suckling group were significantly lower than in the corresponding controls. In contrast, withdrawal of the neurogenic stimulus of suckling for 24 h resulted in an inability of prolactin to block LH release (Fig. 2b). Although a comparison between the lactating and the non-lactating groups provided a means of assessing the influence of suckling/endogenous prolactin on LHRH responsiveness, the model in which exogenous prolactin was used, although it is a heterologous species, clearly shows that prolactin *per se* influences pituitary responsiveness to LHRH during lactation.

Ovarian quiescence during lactation has attracted the attention of several investigators. Our study with subhuman primates demonstrates that during lactation the monkey,

similar to the rat, has a decreased responsiveness to LHRH. Although the ability of LHRH to release LH from the pituitary is reduced if suckling is present, the effect is further accentuated by a single injection of prolactin. Based on our results with the rat, it can be presumed that in the monkey also the inhibitory effect of prolactin on pituitary responsiveness is direct, and not mediated by ovarian or adrenal progesterone. The mechanism and duration of this inhibition is under investigation.

Although others have shown with rats that continued application of the suckling stimulus by litter replacement^{7,8} or injection of prolactin⁹ can prolong lactation considerably, we have observed that a single injection of prolactin can extend the anoestrus period significantly². Recent studies in humans have shown that thyrotropin-releasing hormone (TRH) extends the lactational amenorrhoeic period¹⁰. Whether continued use of TRH would, in addition to releasing prolactin by continuously releasing thyroid-stimulating hormone (TSH), have an adverse effect on thyroid metabolism is a point to be considered. The present study, by demonstrating the possible mechanisms whereby gonadotrophin release is suppressed during lactation, suggests the use of human prolactin to extend the amenorrhoeic condition in lactating women.

The monkey is known to be less responsive to LHRH than the human and the rat¹¹. Consequently, the increase (28–66%) in LH levels observed after administration of LHRH and the maintenance of the response for 20–40 min suggests that we observed a true increase in LH. It has been suggested that there is no apparent difference in the levels of follicle-stimulating hormone and LH between lactating and non-lactating humans¹². With the radioimmunoassay techniques available it may be difficult to pick out differences in the levels of these hormones, particularly when looking for a suppression over the tonic levels. Our study with the monkey, as well as our earlier work with the rat, has shown that withdrawal of the suckling stimulus results in an increase in LH levels over the basal value. Similarly, the use of LHRH has enabled us to show the presence of a clear difference in LH levels of lactating and non-lactating animals.

This study, however, does not exclude the effect of suckling/prolactin directly on the ovarian responsiveness to exogenous gonadotrophins. McNatty *et al.*¹³, using *in vitro* cultures of human granulosa cells, have suggested that prolactin could have an influence on the end-organ responsiveness to exogenous gonadotrophins.

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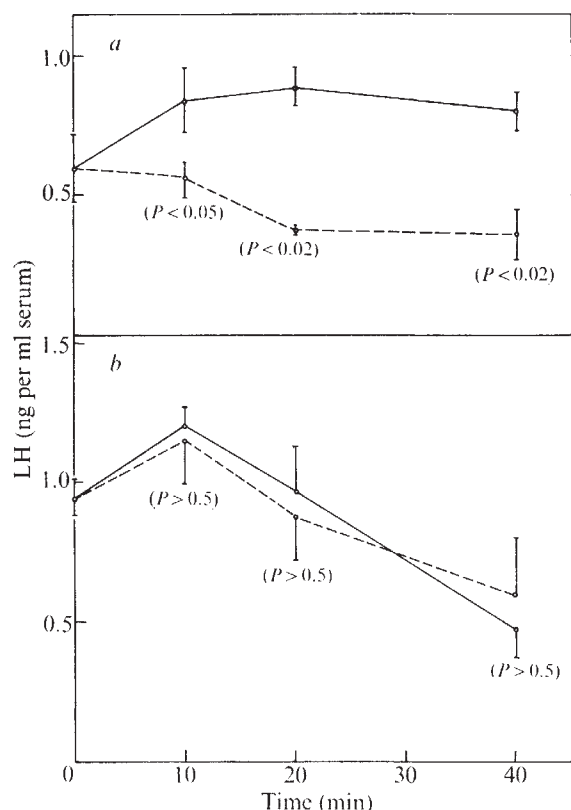
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Fig. 2 Effect of prolactin on pituitary response to LHRH during lactation in the presence (a) and absence of suckling stimulus (b). P values were calculated for values at any given time between the treated and untreated groups. All values > 0.05 were considered to be not significant. —, No prolactin; - - -, plus prolactin.



Bacteria necessary to produce experimental autoimmune myasthenia gravis in acetylcholine receptor-immunised rabbits?

We have¹⁻³ described previously a paralytic disease observed in rabbits immunised with small doses, in Freund's complete adjuvant, of nicotinic cholinergic receptor protein (nAChR) isolated from the electric organ of *Torpedo marmorata*. Decremental muscle response after nerve stimulation and positive effects of anticholinesterase administration, decrease in miniature endplate potential amplitude and increased curare sensitivity were similar to findings in patients suffering from myasthenia gravis. Nicotinic AChR antibody titres above 2–3 mg ml⁻¹ have been found, the observed muscle decrement increasing with increasing antibody titre. Occasionally, variations in electromyographic activity were observed, suggesting denervation. Morphological analysis^{3,4} showed severe destruction of postsynaptic motor endplate areas and reduction of toxin-binding sites, followed by changes at the presynaptic side. A second stage of the disease was characterised by high sensitivity to nAChR injections and by the presence of motor endplates lacking secondary postsynaptic foldings. Our work and that of others (for review see ref. 5) suggests that an immune response to nAChR seems to be involved in the aetiology of experimental autoimmune myasthenia gravis (EAM) just as is suggested for human myasthenia gravis. The role of a humoral and/or cellular immune response and that of the thymus are under discussion^{5,6}. The decremental response to repetitive nerve stimulation may be caused directly and/or indirectly by nAChR antibodies, whereas defects in electromyographic activity such as high frequency discharges and fibrillation action potentials which we have observed may have been caused by other, accompanying mechanisms. This report deals with the role of bacteria in EAM and with the relationship of the antibody titre to the electrophysiological findings.

A sterile-filtered receptor solution produced a low antibody titre, and no decremental response on repetitive stimulation or response to Tensilon was observed. The nAChR used previously¹⁻³ in immunisation which always resulted in animals with EAM was obtained after solubilisation with phosphate buffer containing 1% Triton X-100 and purification by affinity and ion-exchange chromatography. Sodium azide (0.02%) was added to the purified receptor to prevent further growth of bacteria. About 30 rabbits were immunised with such preparations, all of which showed typical symptoms of EAM.

We have introduced a sterile filtration step (Millipore filters, pore size 0.22 µm) after Triton X-100 solubilisation to reduce bacteria present from 4,000–12,000 to 30 ml⁻¹.

Table 1 Relationship between nAChR antibody titre and decremental muscle response after repetitive stimulation of peroneal nerve

Rabbit no.	Antibody titre (mg ml ⁻¹)	Decrement (%)
1	2.9	56
2	4.9	25
3	6.7	62
4	3.0	37
5	3.4	40
6	0.7	5
7	1.2	8
8	0.7	6
9	0.7	6
10	0.5	7

Antibody titres and decrement in rabbits immunised with a non-sterile (1–5) and a sterile (6–10) receptor preparation.

The receptor obtained had chemical and physical properties similar to those of material obtained previously, except for a considerably lower antigenicity, as expressed in obtained antibody titre in otherwise identical conditions. Rabbits immunised with a 'sterile' receptor show greater variability in the onset time for the disease, but the clinical picture observed is close to those animals observed previously. The electrophysiological investigations revealed only a very small decrement, about 5–10%, which could not be responsible for the severe visible symptoms in the examined rabbits. An analysis of the receptor antibody titres (rocket immunoelectrophoresis) showed that these are rather low, below 1.2 mg ml⁻¹. A return to receptor purification without sterile filtration resulted in rabbits with high antibody titres and greater decrement. Results from ten rabbits are summarised in Table 1.

We conclude that so called 'EAM rabbits' may suffer from at least two different kinds of defects: one at the neuromuscular junction, which may be of the kind observed in myasthenia gravis (this defect seems to be clearly correlated with the titre of circulating antibody to nAChR); and one, responsible for the severe symptoms visible in rabbits immunised with receptor isolated after sterile filtration, which seems to involve denervation rather than neuromuscular deficiency, revealed in the electromyographic investigation as high frequency discharges and fibrillation action potentials³. These effects were observed also at low nAChR antibody titres.

We have found therefore that besides 'myasthenic symptoms' EAM rabbits seem to have another type of weakness, not correlated with high antibody titre. We may be dealing with a potentiation of nAChR antigenicity, but a specific effect of a bacteria—for example, due to binding to and/or transformation of nAChR—may well be the explanation. It is also possible that the filtration step removes non-bacterial material—for example, aggregated proteins. We have, however, not noticed a lower receptor yield in our final preparations.

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Cyclic AMP and cyclic GMP may play opposing roles in influencing force of contraction in mammalian myocardium

CYCLIC AMP and cyclic GMP have been suggested to play opposing regulatory roles in several biological systems¹. Supporting evidence for the yin yang hypothesis of opposing biological regulation has been obtained in sympathetic ganglia^{2,3} and pyramidal neurones in the rat cerebral cortex⁴. In the mammalian heart, the role of cyclic AMP in mediating the positive inotropic response to catecholamines was advanced by the observation that the inotropic effect was preceded by an increase

in cyclic AMP levels⁵. On the other hand, the levels of cyclic GMP were found to be increased after cholinergic stimulation⁶. In frog, oscillations of cyclic AMP⁷ and cyclic changes in the levels of cyclic AMP and cyclic GMP⁸ during the cardiac cycle have been reported. The importance of cyclic AMP for the inotropic effects of catecholamines was confirmed by the observation that the dibutyryl derivative of cyclic AMP exerted a positive inotropic effect in cat papillary muscles⁹ and in the isolated perfused hearts from rabbits, rats and guinea pigs¹⁰. I now report a direct negative inotropic effect of 8-Br-cyclic GMP on force of contraction (F_c) in rat auricles and papillary muscles from cats.

Rats and cats were anaesthetised with ether and either auricles or papillary muscles were dissected from the heart and mounted in a muscle chamber for recording mechanical activity as described previously¹¹. Left auricles were driven electrically at 2 Hz and cat papillary muscles at 0.2 Hz. Drugs (all cyclic nucleotides from Boehringer/Mannheim) were freshly dissolved in distilled water and 0.1 ml was injected into the muscle chamber containing 5 ml of Tyrode solution (composition in mM: NaCl, 136.9; KCl, 5.4; MgCl₂, 1.05; CaCl₂, 1.8; NaH₂PO₄, 0.42; NaHCO₃, 11.9; glucose, 5.5) which was gassed with 95% CO₂-5% O₂ and heated to 35 °C.

Figure 1 shows the influence of 8-Br-cyclic GMP on F_c in electrically driven left auricles and on frequency of spontaneously beating right auricles isolated from rat hearts. 8-Br-cyclic GMP exerted a concentration-dependent negative inotropic effect; when the preparation was washed three times in drug-free solution, the negative inotropic effect was partially reversible, but F_c did not return fully to its previous control value within 60 min. The frequency of spontaneously beating right auricles was not significantly affected by 8-Br-cyclic GMP within the whole concentration range. Control experiments showed that the negative inotropic effect of 8-Br-cyclic GMP was not influenced when the preparations were pretreated with 5×10^{-6} M

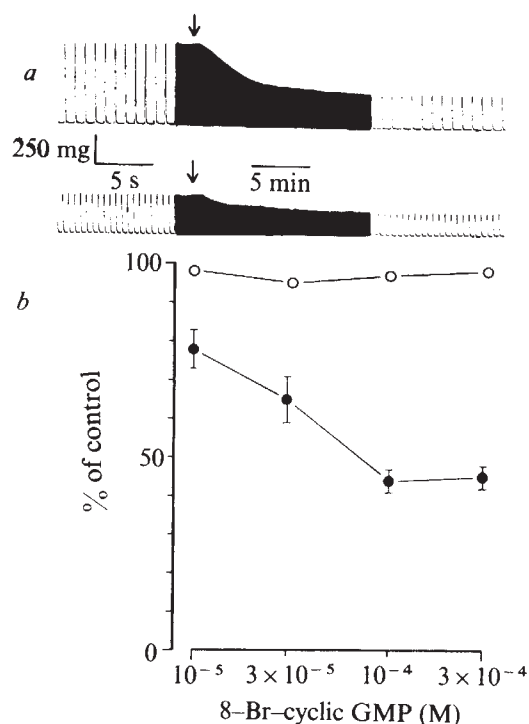


Fig. 1 Effect of 8-Br-cyclic GMP on force of contraction (F_c) and frequency in isolated auricles from rat hearts. F_c was determined in electrically driven left auricles, frequency in spontaneously beating right auricles. *a*, Original traces of a left (upper) and a right (lower) auricle in response to 3×10^{-4} M 8-Br-cyclic GMP. Addition of drug is indicated by the arrows. *b*, Concentration-response relationships. Each concentration was tested in individual preparations. Symbols represent means $\pm$ s.e. of 8–16 preparations. Values of s.e. for the frequency of contraction are smaller than the size of the symbols used and are therefore omitted. $\circ$, Frequency; $\bullet$, F_c .

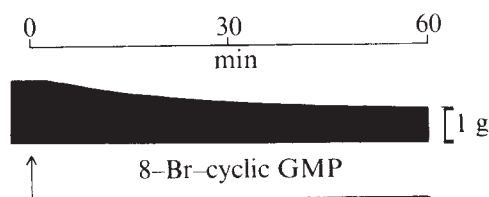


Fig. 2 Effect of 3×10^{-4} M 8-Br-cyclic GMP on F_c in a cat papillary muscle.

atropine sulphate. 10^{-3} M cyclic GMP (not substituted) or 10^{-3} M NaBr did not affect F_c . Figure 2 shows the influence of 8-Br-cyclic GMP on F_c in a cat papillary muscle (mean $\pm$ s.e. of four experiments: decrease of F_c to $67.7 \pm 3.7\%$ of control values). The negative inotropic effect of 8-Br-cyclic GMP was fully reversible within 60 min when the preparation was washed three times in drug-free solution. For comparison, the effects of 8-Br-cyclic AMP were also studied. In papillary muscles from cats, 8-Br-cyclic AMP did not affect F_c ; however, after pretreatment with the strong phosphodiesterase inhibitor papaverine (2×10^{-5} M) a positive inotropic effect was observed (Fig. 3; mean $\pm$ s.e. of six experiments: increase of F_c to $180 \pm 22.1\%$ of control values before addition of 8-Br-cyclic AMP).

In atrial tissue, studies with cyclic AMP and its derivatives are complicated by the fact that the breakdown products of cyclic AMP or cyclic AMP itself—if applied from outside the cell—have a strong negative inotropic effect in auricles¹². In rat left auricles, 8-Br-cyclic AMP caused a sharp decline of F_c that was followed by a second component of a slowly developing increase in F_c (Fig. 4). This second component seems to correspond to the positive inotropic effect seen in cat papillary muscles (Fig. 3), since it was augmented after pretreatment with 2×10^{-5} M papaverine.

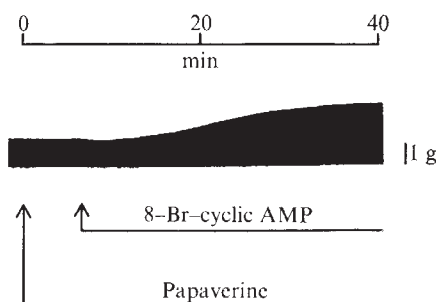


Fig. 3 Effect of 3×10^{-4} M 8-Br-cyclic AMP in a papillary muscle from a reserpinised cat (Serpasil, Ciba, 5 mg kg⁻¹, 18 h before the experiment) in the presence of 2×10^{-5} M papaverine. Both papaverine and 8-Br-cyclic AMP alone did not affect F_c but only if given in combination. The same positive inotropic effect was obtained when 8-Br-cyclic AMP was added first and then papaverine. Papaverine itself (2×10^{-5} M) exerts a slight positive inotropic effect in papillary muscles from non-reserpinised cats that is accompanied by an increase in the slow inward current as measured by the voltage clamp technique (H.N., to be published). The positive inotropic effect disappears after pretreatment with papaverine has a negative inotropic effect; this corresponds to the findings of Schneider *et al.*¹⁴ who reported a depression of Ca-dependent action potentials in the guinea pig heart with 4×10^{-4} M papaverine. Papaverine (2×10^{-5} M) was given in preference to other drugs because it strongly inhibits the phosphodiesterase activity in the heart but does not affect F_c in cat papillary muscles provided that any adrenergic influences are excluded. Cats were reserpinised to exclude catecholamine effects due to either papaverine or 8-Br-cyclic AMP. This is an appropriate and widely used method to exclude any interference by noradrenaline which may be eventually released by the use of some drugs. The possibility that 8-Br-cyclic AMP releases noradrenaline is not excluded; papaverine, indeed, is able to release endogenously stored catecholamines¹⁵.

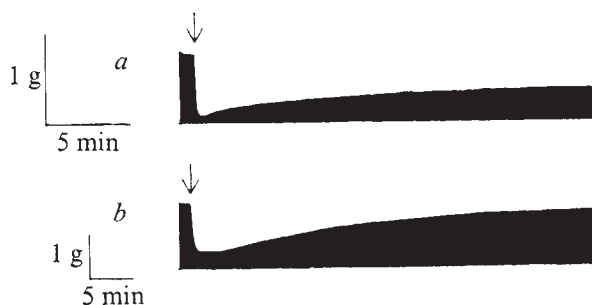


Fig. 4 Effect of 3×10^{-4} M 8-Br-cyclic AMP without (a) and in the presence of 2×10^{-5} M papaverine (b) in the same preparation (rat left auricle). Papaverine alone had a positive inotropic effect: control F_c in (b) before addition of 8-Br-cyclic AMP, indicated by the arrows, was about twice as big as in (a). The initial negative inotropic effect of 8-Br-cyclic AMP in rat atrial muscle most probably resulted from 8-Br-cyclic AMP *per se*, if applied from outside the cell, since 10^{-3} M 8-Br-5' AMP (Schwarz/Mann)—the non-cyclic breakdown product of 8-Br-cyclic AMP—did not produce either the negative or the positive inotropic effect.

These findings indicate a positive inotropic action of 8-Br-cyclic AMP and the sum of results therefore suggests an opposite influence of cyclic GMP and cyclic AMP on F_c in atrial as well as in ventricular myocardium. In contrast to the negative inotropic action of 8-Br-cyclic GMP in rat auricles, the pacemaker activity was virtually unaffected by the drug. This points to a difference in the action of acetylcholine on rat atrial muscle. Voltage clamp experiments have shown that the negative inotropic effect of acetylcholine in mammalian atrial muscle can be explained by an increase in potassium conductance at lower doses and, additionally, by a decrease in calcium conductance at higher doses¹³. It remains open whether similar conductance changes are brought about by the action of cyclic GMP and can be related to the corresponding changes in force of contraction.

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Opposing effects of cyclic AMP and cyclic GMP on protein phosphorylation in tubulin preparations

Cyclic AMP and cyclic GMP have been postulated to be in certain cases, antagonistic intracellular messengers of many hormones and neurotransmitters acting at the cell membrane¹. At least part of their antagonism may be the result of

their ability to affect, in opposite directions, specific protein kinases and phosphates which work on common substrates (enzymes, structural proteins). Cyclic AMP and cyclic GMP have been found to activate, and the former also to inhibit, specific protein kinases^{2–5}; cyclic AMP can activate or inhibit^{3–8} specific protein phosphates, and both cyclic nucleotide-sensitive protein kinases and protein phosphates have been isolated forming a complex with their substrate⁹. Some of these effects of cyclic nucleotides, however, have been found to depend on the type and concentration (not necessarily physiological) of the ion used in the assay⁵ and no direct evidence exists to show that one of the cyclic nucleotides opposes the effects of the other on protein phosphorylation. We report here that physiological concentrations (10^{-7} M– 10^{-9} M) of cyclic GMP oppose the cyclic AMP (10^{-6} M) dependent phosphorylation of a soluble, perhaps tubulin-related brain protein, possibly by activation of a phosphoprotein phosphatase.

The enzyme preparation we used is the eluate of the second of two sequential isodeacetylcolchicine-deacetylcolchicine (I-DAC) affinity columns used⁹ to purify tubulin from 100,000g rat brain supernatants (see legend to Table 1). Previous studies have shown that tubulin purified by these and other methods, contains endogenous protein

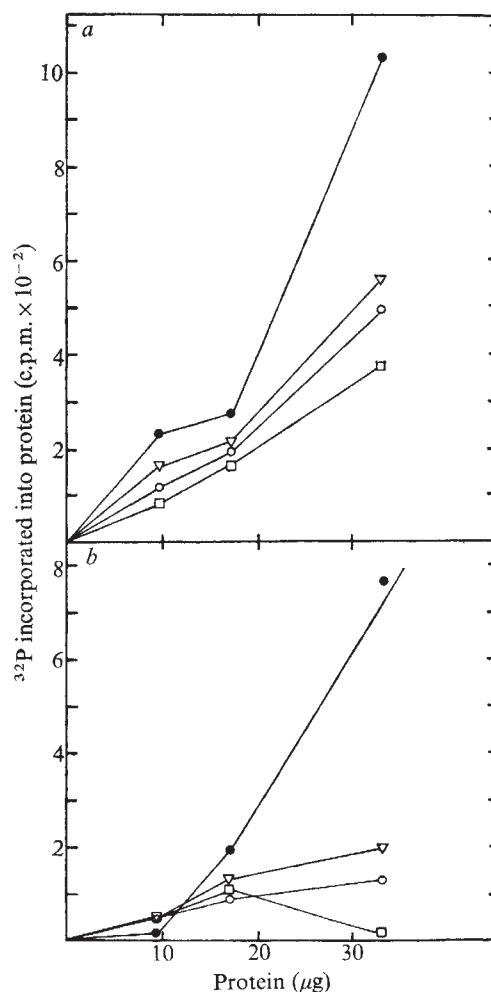


Fig. 1 Effect of cyclic GMP on endogenous protein phosphorylation as a function of the concentration of protein. Data presented correspond to the same experiment: a, whole protein kinase; b, cyclic AMP-stimulated protein kinase. Preparation of the kinase and its assay (5 min, 37 °C) were as described in Tables 1 and 2. The following additions were made to the assay mixture: (●) 2.5×10^{-6} M cyclic AMP, (△) 2.5×10^{-6} M cyclic AMP plus 2.5×10^{-7} M cyclic GMP, (□) 2.5×10^{-6} M cyclic AMP plus 2.5×10^{-8} M cyclic GMP, (○) 2.5×10^{-6} M cyclic AMP plus 2.5×10^{-9} M cyclic GMP.

kinase activity¹⁰⁻¹³ which specifically phosphorylates high molecular weight proteins closely associated with tubulin^{9,14}. Furthermore, essentially all of the kinase activity in such preparations (including that observed in the absence of added cyclic AMP) is fundamentally cyclic AMP dependent since total suppression of activity occurs⁹ on addition of a specific muscle protein kinase modulator¹⁵.

Phosphorylation of endogenous protein in the various colchicine-Agarose eluate fractions is strongly opposed by very

cyclic GMP vary between 10^{-7} and 10^{-9} M. Although there is some experimental variability, the data from many experiments indicate that cyclic GMP concentrations above or below 10^{-8} M are usually less effective (Tables 1 and 2A).

The inhibitory effect of cyclic GMP and the relative effectiveness of varying concentrations of this nucleotide are maintained over a relatively wide range of protein concentration in each individual experiment, although the total phosphorylation varies from preparation to preparation (for

Table 1 Cyclic GMP inhibition of protein kinase activity in various fractions of a rat brain tubulin preparation

Addition	³² P incorporated into protein (c.p.m.)					
	Column effluent (fraction number)					
	1	2	3	4	5	6
None	0	0	0	120	450	480
2.5×10^{-6} M cyclic AMP	0	50	2,580	1,850	830	290
2.5×10^{-6} M cyclic AMP + 2.5×10^{-7} M cyclic GMP	220	100	60	260	590	380
2.5×10^{-6} M cyclic AMP + 2.5×10^{-8} M cyclic GMP	250	320	0	80	0	410
2.5×10^{-6} M cyclic AMP + 2.5×10^{-9} M cyclic GMP	180	0	0	160	370	400

Cyclic GMP inhibition of protein kinase activity in various fractions of a rat brain tubulin preparation chromatographed on a I-DAC-Agarose affinity column. Rat brains (10 g) were homogenised in 10 ml of 10 mM sodium phosphate, pH 7.65, containing 5 mM MgCl₂ and 1 mM GTP (buffer A). The 100,000g (1 h, 4 °C) supernatant of the homogenate was applied (4–10 °C) on a 2 ml I-DAC-Agarose column⁹ (containing 160 nmol of bound ligand) equilibrated with buffer A. The column was washed thoroughly (50 ml) with buffer A before the protein was eluted with the same buffer containing 0.1 M NaCl (4 °C). The eluate was desalted on a 40-ml Sephadex G-25 (coarse grade) column equilibrated with buffer A. The protein was then reappplied to the same I-DAC column after washing with 7 M guanidine-HCl followed by equilibration with buffer A. The 0.1 M NaCl eluate of this column was collected in sequential 0.7-ml fractions, each of which was tested for protein kinase activity using the endogenous protein present in the sample as substrate. The enzymatic assay mixture contained, in a total final volume of 200 µl, 50 µmol potassium phosphate, pH 7.5, 5 µmol MnCl₂, 2 nmol of unlabelled ATP, 2×10^6 c.p.m. of γ -³²P-ATP and 50 µl of sample (column eluent) which contained 5 mM MgCl₂, 1 mM GTP and 0.1 M NaCl. The samples were incubated for 5 min at 37 °C and the reaction was stopped by the addition of 3 ml of ice-cold 20% trichloroacetic acid plus 300 µg of serum albumin per 200 µl of assay mixture. The values have been corrected for blanks (880 c.p.m.), which were determined by adding the radioactivity after stopping the reaction. The ³²P incorporated into protein was determined (liquid scintillation) in the second 20% trichloroacetic acid precipitate after dissolving in 0.5 ml of 1 M NaOH. Values are averages of duplicate replications.

low, physiological concentrations of cyclic GMP (Table 1, fractions 3, 4 and 5). Although such inhibition is most marked when exogenous cyclic AMP is added, significant inhibition occurs reproducibly even in the absence of added cyclic AMP. The inhibition occurs at physiological pH (7.5) and temperature (37 °C), whether Mg²⁺ (5 mM), Mn²⁺ (5 mM), or both are used and the optimal concentrations of

example, we have observed endogenous phosphorylation-dephosphorylation in preparations containing as little as 1 µg of protein per assay) (Fig. 1).

During the early stages of the kinase assay (for example, 1 min) the inhibitory effect of cyclic GMP is usually not apparent (Table 2A). As incubation progresses (from 5 to 10 min), with the concomitant increase in the quantity of

Table 2 Effect of length of incubation on the cyclic GMP inhibition of cyclic AMP-stimulated protein kinase activity of tubulin preparations

Cyclic nucleotide added	³² P (c.p.m.) incorporated into protein		
	Incubation time (min)		
	1	2	5
Experiment A			
None	130	220	540
2.5×10^{-6} M cyclic AMP	380	1,000	2,570
2.5×10^{-6} M cyclic AMP + 2.5×10^{-6} M cyclic GMP	240	640	1,640
2.5×10^{-6} M cyclic AMP + 2.5×10^{-7} M cyclic GMP	570	430	1,410
2.5×10^{-6} M cyclic AMP + 2.5×10^{-8} M cyclic GMP	430	780	180
2.5×10^{-6} M cyclic AMP + 2.5×10^{-9} M cyclic GMP	360	660	400
2.5×10^{-6} M cyclic AMP + 2.5×10^{-10} M cyclic GMP	310	570	740
2.5×10^{-6} M cyclic AMP + 2.5×10^{-11} M cyclic GMP	470	740	500
	5	10	15
Experiment B			
None	1,180	800	160
2.5×10^{-6} M cyclic AMP	2,260	910	340
2.5×10^{-6} M cyclic AMP + 2.5×10^{-7} M cyclic GMP	710	1,020	20
2.5×10^{-6} M cyclic AMP + 2.5×10^{-8} M cyclic GMP	1,240	0	0
2.5×10^{-6} M cyclic AMP + 2.5×10^{-9} M cyclic GMP	440	550	50

Effect of length of incubation on the cyclic GMP inhibition of cyclic AMP-stimulated protein kinase activity of tubulin preparations. The two independent experiments shown were carried out with protein purified on two sequential I-DAC affinity columns, as described in Table 1. The eluent samples of the second column were pooled before assay. Kinase activity was determined as described in Table 1, using the endogenous protein as the substrate (15 µg). Results essentially similar to those described here have been obtained in at least 20 separate experiments. The data have been corrected for blanks (490 c.p.m. in A, 1,100 c.p.m. in B).

phosphorylated protein, the effect of cyclic GMP becomes apparent. With progressively longer periods of incubation, however, the effects of cyclic GMP become less obvious, or are obscured by the obvious dephosphorylation which occurs spontaneously in all conditions (Table 2B). The kinetic data are most consistent with a cyclic GMP-induced acceleration of a protein dephosphorylation reaction. Addition of adenosine thiotriphosphate (as a carrier) together with γ - 32 P-ATP decreases greatly the rate of spontaneous dephosphorylation, consistent with the presence of protein phosphatases¹⁶.

Although the kinase activity in the tubulin preparation can effectively phosphorylate exogenously added proteins such as histone (lysine enriched, f1), such phosphorylation is not opposed by cyclic GMP (Table 3). On the other hand, phosphorylation of aged (24 h, -20°C) colchicine-Agarose eluates (I-DAC), which also occurs readily in the absence of cyclic AMP, is clearly inhibited by cyclic GMP in the absence, but not presence, of cyclic AMP. This suggests that the cyclic GMP-enhanced dephosphorylation may be restricted to certain specific sites of natural protein substrates. These latter sites are probably not the only ones available for phosphorylation in the aged natural substrate when the protein kinase activity is fully expressed (that is, in the presence of cyclic AMP).

selves phosphorylated by a cyclic AMP-dependent protein kinase. We will not speculate further on the possible mechanism of this cyclic GMP effect. These observations, together with earlier studies demonstrating that the kinase activity of the tubulin preparation used here is completely cyclic AMP dependent⁹, and that the proteins phosphorylated (*in vivo* as well as *in vitro*) are specific high molecular weight proteins closely associated with the 36S tubulin molecule^{9,14}, may be pertinent to understanding the regulation of the structure and function of tubulin and microtubules. The specific cyclic GMP effect described here may also have broader significance with respect to the biochemical actions of this cyclic nucleotide. The low levels of protein phosphatase activity in preparations of tubulin obtained by two cycles of polymerisation-depolymerisation may reflect a weak association of the protein phosphatase with tubulin, or may be uniquely related to the specific method of purification. The fact, however, that certain colchicine derivatives, such as that used in the affinity adsorbent used here, inhibit bacterial and animal non-protein alkaline phosphatases as well as vegetable non-protein acid phosphatases¹⁷ opens the possibility that the I-DAC affinity column may also retain protein phosphatases physiologically unrelated with tubulin or functionally related proteins.

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Table 3 Effect of cyclic GMP on the phosphorylation of endogenous and exogenous substrates by the eluate of I-DAC-Agarose affinity columns

Cyclic nucleotide additions	³² P (c.p.m.) incorporated into protein		
	Fresh I-DAC protein	Aged I-DAC protein	Histone*
None	700	4,070	3,210
5×10^{-6} M cyclic AMP	3,770	4,580	5,120
5×10^{-7} M cyclic GMP	780	2,920	3,980
5×10^{-8} M cyclic GMP	600	1,900	5,560
5×10^{-9} M cyclic GMP	580	3,820	6,320
5×10^{-6} M cyclic AMP + 5×10^{-7} M cyclic GMP	1,270	3,850	5,490
5×10^{-6} M cyclic AMP + 5×10^{-8} M cyclic GMP	2,000	4,390	7,620
5×10^{-6} M cyclic AMP + 5×10^{-9} M cyclic GMP	1,120	4,110	6,810

Effect of cyclic GMP on the phosphorylation of endogenous and exogenous substrates by the eluate of I-DAC-Agarose affinity columns. The kinase preparation described in Table 2 was incubated for 5 min (37°C) with no other addition (fresh I-DAC protein), with an aged, desalted eluent fraction (10 μg of protein) which had been stored at -20°C for 24 h (aged I-DAC protein) or with 10 μg of histone. Assay conditions were as described in Table 1.

*Histone type V (Sigma), lysine-rich subgroup f1.

The effects of cyclic GMP cannot be explained on the basis of competition or direct antagonism of a cyclic AMP effect since they can occur even in the absence of cyclic AMP, they clearly follow an initial phosphorylation reaction, the inhibition is much less effective at the highest concentrations of cyclic GMP tested, and direct binding studies using ^3H -labelled cyclic AMP (10^{-8} M) show no competition of binding with cyclic GMP concentrations varying from 10^{-7} M to 10^{-9} M. With regard to nucleotide specificity, it is important that the cyclic GMP (10^{-7} – 10^{-9} M) effect on protein phosphorylation is observed in reaction mixtures containing much higher concentrations of cyclic AMP (10^{-6} M), ATP (10^{-6} M) and GTP (10^{-4} M). In certain types of experiments it has been observed consistently that prolonged incubation of the kinase assay mixture results in a slow cyclic GMP-stimulated phosphorylation, suggesting the possible additional existence in this system of a specific cyclic GMP-dependent kinase which can complicate observations of the specific phosphatase effect described here (Table 3). For this reason, careful attention to the time course of the assay at a given protein concentration is critical for the demonstration of the cyclic GMP-enhanced dephosphorylation reaction.

These studies suggest the existence of a highly specific, cyclic GMP-sensitive phosphoprotein phosphatase which acts on specific sites of natural substrates which are them-

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Structural properties of rat serum proteins which correlate with their degradative rates *in vivo*

SERUM proteins^{1,2} like intracellular proteins³⁻⁷, are continually being degraded and replaced at characteristic rates. Although there is appreciable evidence that structural alterations can influence the half lives of serum proteins⁸⁻¹¹, the features of serum proteins which normally influence their degradative rates are unknown. The present experiments were undertaken to learn more about the factors influencing the degradation of serum proteins and to explore any possible relationship of this process to the catabolism of intracellular proteins.

There is now strong evidence that the degradative rates of intracellular proteins are determined to a large extent by their conformations^{3,6}. For example, in both animal and bacterial cells, the catabolic rates of different proteins correlate with their relative sensitivities *in vitro* to purified endoproteases³⁻⁷ and to lysosomal extracts¹²⁻¹⁴. In addition two structural features of normal proteins have been shown to correlate with their half lives in a wide variety of organisms and tissues: (1) proteins with large subunits tend to be degraded more rapidly on the average than those with small subunits^{3-7,15}, and (2) proteins with acidic isoelectric points tend to be degraded more rapidly than proteins with neutral or basic isoelectric points¹⁶.

Analogous studies of the possible relationship between the degradative rates of serum proteins and these structural properties have not been reported. We now present evidence that the catabolism of serum protein shows two of the characteristics previously reported for degradation of intracellular proteins: (1) the rates of catabolism of rat serum proteins *in vivo* correlate with their relative sensitivity *in vitro* to a wide variety of endoproteases, and (2) the more acidic serum proteins turn over *in vivo* more rapidly than neutral or basic ones. In contrast to findings with intracellular proteins, no relationship was found between the molecular weights of serum proteins and their half lives *in vivo*.

To test whether half lives of serum proteins are related to their sensitivity to proteolytic attack, we used the double-isotope approach of Arias *et al.*¹⁷ to differentiate serum proteins which turn over rapidly from those which turn over slowly *in vivo*. This same technique was used previously to establish a correlation between degradative rates and protease sensitivity for intracellular proteins⁷. ¹⁴C-leucine was administered to a rat 4 d before an injection of ³H-leucine, and the animal was killed 4-12 h after the second injection. The ¹⁴C remaining in protein after 4 d is primarily contained in the more stable proteins, while the ³H is found preferentially in proteins that turn over rapidly^{3,7,17,18}.

We then compared the susceptibility to various proteases of proteins having shorter *in vivo* half lives (enriched for ³H) and those that are more stable (enriched for ¹⁴C). As shown in Fig. 1, serum proteins rapidly degraded *in vivo* were more susceptible to digestion at pH 7.2 by Pronase (a,f), trypsin (b) and chymotrypsin (c) and at pH 4.0 by the acidic proteases, pepsin (d) and lysosomal cathepsins

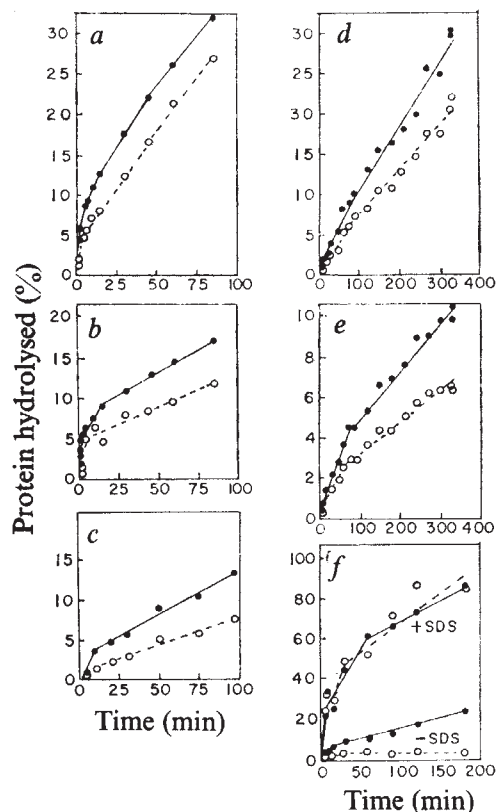


Fig. 1 Comparison of the susceptibility of stable and rapidly turning-over serum proteins to proteolytic enzymes. To label these fractions preferentially, 100 μ Ci of ¹⁴C-leucine was administered intraperitoneally to a male rat (125 g). Four days later, the same animal was injected with 500 μ Ci of ³H-leucine and killed 4 or 12 h thereafter. Consequently, the ¹⁴C is found primarily in proteins with relatively long half lives (○) while ³H-proteins (●) are enriched for more labile polypeptides. To prepare the serum, the rats were anaesthetised with ether, blood (about 5 ml) was collected from the aorta into a heparinised tube, and cells were separated by centrifugation at 100g for 10 min. The clear supernatant containing the serum proteins was dialysed against phosphate-buffered saline (pH 7.2, ref. 7) (for experiments a-c and f) or 100 mM citrate buffer (pH 4.0) (for experiments d and e) to remove free amino acids. The procedure for incubation with proteases, and the sources for all materials have been reported previously⁷. Each incubation mixture contained approximately 5 mg of serum protein with a specific activity of about 70,000 d.p.m. ³H and 5,000 d.p.m. ¹⁴C per mg. Enzymes were added at the following concentrations: a, Pronase, 100 μ g ml⁻¹; b, trypsin, 100 μ g ml⁻¹; c, chymotrypsin, 1 mg ml⁻¹; d, pepsin, 50 μ g ml⁻¹; e, crude lysosomal fraction (prepared as described in (31)), 10 mg ml⁻¹; f, Pronase, 50 μ g ml⁻¹. Conformations of serum proteins were disrupted in (f) by addition of 1% SDS and 50 mM dithiothreitol (final concentration) and boiling for 5 min. (Pronase is at least partially active in SDS.) Protein hydrolysis is expressed as radioactivity (d.p.m.) initially in protein that is converted to a trichloroacetic-acid soluble form.

from rat liver (e). Similar results were obtained with three separate preparations of double-labelled proteins from rat serum. These findings are analogous to those obtained earlier with intracellular proteins^{3-7,12-14}, although the serum proteins on the average appeared much less susceptible to trypsin and chymotrypsin than were liver proteins (J.F.D. and A.L.G., unpublished).

These results indicated that the rates of catabolism of serum proteins are related to their inherent susceptibility to proteolytic attack, which is presumably a function of the proteins' native conformations. In fact, the susceptibility of both rapidly and slowly turning-over proteins to digestion by Pronase increased markedly when the native conformations of the proteins were destroyed by boiling in sodium dodecyl sulphate (SDS). Following such treatment, the correlation between degradative rates *in vivo* and susceptibility to Pronase was abolished (Fig. 1f).

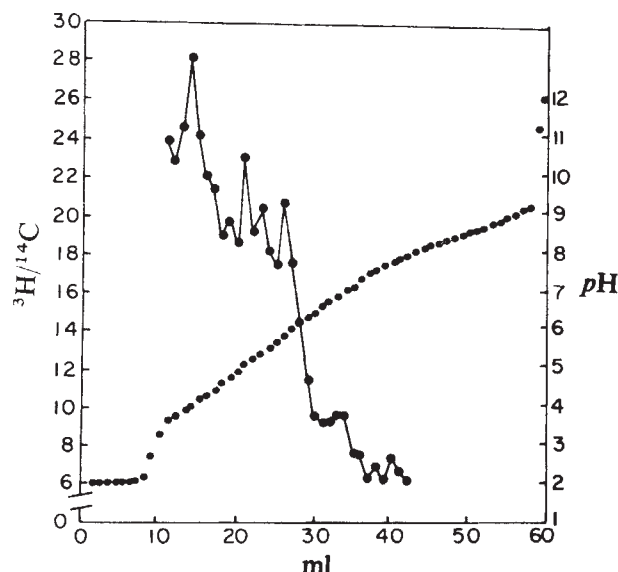


Fig. 2 Comparison of degradative rates of rat serum proteins with different isoelectric points. Double-labelled serum proteins were prepared as described in Fig. 1 and separated by isoelectric focusing as reported previously¹⁶. A high $^3\text{H}/^{14}\text{C}$ ratio indicates more rapid turnover of the fraction¹⁷. Radioactivity in each fraction was determined as described previously. Ratios are not plotted for fractions containing less than 200 d.p.m. for each isotope. The variation in $^3\text{H}/^{14}\text{C}$ ratios inherent in these experimental protocols has been reported previously¹⁶. Fractions from isoelectric focusing contained between 0 and 10,000 d.p.m. of ^3H and 0 and 500 d.p.m. of ^{14}C . The correlation between $^3\text{H}/^{14}\text{C}$ and pH is highly significant ($r = -0.957$, $P < 0.01$). pH, ●●●●; $^3\text{H}/^{14}\text{C}$, ●—●.

We used the same labelling procedures to compare the degradative rates *in vivo* of serum components differing in their isoelectric points. For any protein, the amount of isotope remaining after 4 d (^{14}C) relative to the amount of isotope initially incorporated (^3H) reflects its rate of degradation. Thus, proteins that are synthesised and degraded rapidly will have high $^3\text{H}/^{14}\text{C}$ ratios, as has been discussed critically elsewhere^{5,7,17,18}. The double-labelled serum proteins were then separated according to their isoelectric points by isoelectric focusing¹⁶.

The more acidic proteins consistently had higher $^3\text{H}/^{14}\text{C}$ ratios ($r = 0.957$, $P < 0.01$). Thus on the average they are degraded more rapidly than the less acidic ones. Qualitatively similar results were obtained with three separate preparations of double-labelled proteins from rats and with two preparations from mice. Thus, a similar correlation between degradative rates and isoelectric points holds for serum proteins (Fig. 2) as for intracellular proteins, even though serum was found to contain much fewer basic proteins than most mammalian tissues (J. F. D. and A.L.G., unpublished observations).

Many earlier studies have demonstrated a correlation between degradative rates of intracellular proteins and their subunit molecular weight as determined by SDS-acrylamide gel electrophoresis^{5-7,13}. But when double-labelled serum proteins were separated by SDS gel electrophoresis, no relationship was found between subunit molecular weight and degradative rates (Fig. 3). These findings differ from those obtained with all mammalian tissues tested. These negative results are complicated by the fact that most serum proteins are glycoproteins which have been shown to migrate more slowly than expected in SDS gels primarily because of a decrease in detergent binding^{19,20}. Since glycoproteins should behave less anomalously during Sephadex gel filtration in the absence of SDS, we have also fractionated serum proteins according to their multimeric size by Sephadex chromatography. For intracellular proteins multimer molecular weights also correlate with half lives¹,

since larger multimers tend to be composed of larger subunits¹⁵. On gel filtration of the serum proteins, however, no relationship was found between molecular weight and degradative rate (Fig. 4).

Thus degradative rates of serum proteins, unlike those of intracellular proteins, do not correlate with their molecular weights (Fig. 3 and 4). Larger intracellular polypeptides have been shown in general to be more sensitive to proteases *in vitro* than smaller ones, and this relationship has been suggested to be the basis for the observed correlation between molecular weight and *in vivo* degradative rates⁷. It is possible that large and small serum components

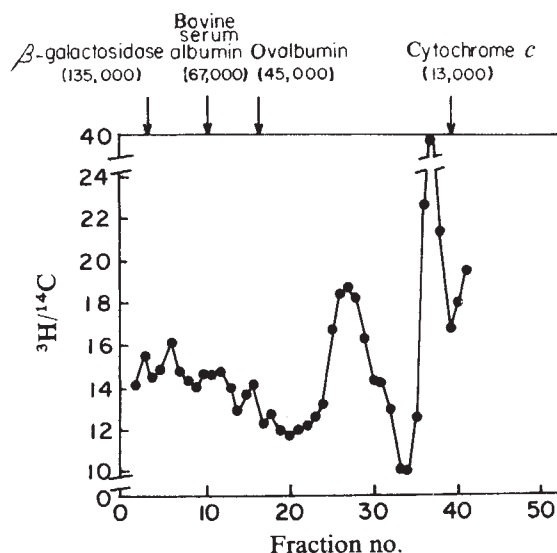


Fig. 3 Relative degradative rates of rat serum polypeptides of different molecular weights. Double-labelled serum proteins were prepared as described in Fig. 1 and separated by SDS-acrylamide gel electrophoresis. A high $^3\text{H}/^{14}\text{C}$ ratio indicates more rapid turnover of the fraction. The electrophoresis was performed in large diameter gels, which were sliced and counted as described earlier⁷. Electrophoretic fractions contained between 0 and 14,000 d.p.m. ^3H and 0 and 1,200 d.p.m. ^{14}C . Ratios are not plotted for fractions containing less than 100 d.p.m. of each isotope. The variation in $^3\text{H}/^{14}\text{C}$ ratios inherent in these methods has been reported previously⁷.

do not differ in susceptibility to proteases. On the other hand, physiological processes are known that can cause the selective loss of small polypeptides from serum and may therefore obscure any general relationship that may exist between protein size and degradative rates. For instance, smaller proteins are lost especially rapidly from the circulation due to ultrafiltration in the renal glomeruli as well as in other capillary beds²². In addition, specific degradative mechanisms appear responsible for the rapid catabolism of small peptide hormones⁷. Thus the reasons and significance of this lack of a size correlation for serum proteins are unclear.

A variety of studies have indicated that the pathway of catabolism of serum proteins involves pinocytosis and subsequent appearance of the proteins in lysosomes^{8,9}. Pinocytosis has been generally assumed to be the rate-limiting step in the degradation of serum proteins. This conclusion is based primarily on studies of the fate of injected enzymes^{23,24} or specific labelled serum proteins^{8,25-27}. The present findings on the turnover of native serum proteins in normal conditions suggest alternative possibilities. Since the degradative rates of serum proteins *in vivo* correlate with their relative susceptibility to a variety of proteases (Fig. 1), the rate-limiting step in the catabolism of most serum proteins may be an initial proteolytic cleavage within the circulation followed by pinocytosis of the partially degraded protein. A rate-limiting proteolytic cleavage also

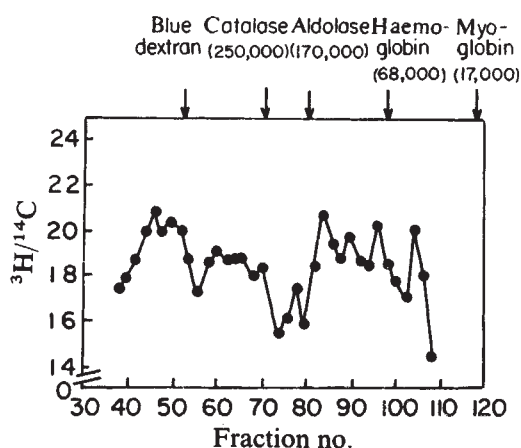


Fig. 4 Comparison of the degradative rates of serum proteins of different multimer size in the absence of SDS. Double-labelled serum proteins were prepared as in Fig. 1 and separated by Sephadex G-150. The protein fractionation and counting of radioactivity was carried out as reported earlier^{7,16}. Chromatographic fractions contained between 0 and 50,000 d.p.m. ³H and 0 and 2,000 d.p.m. ¹⁴C. Ratios are not plotted for fractions containing less than 100 d.p.m. of each isotope.

might possibly occur within the secondary lysosome. According to this hypothesis, proteins that are relatively resistant to lysosomal proteases might then be released and re-enter the circulation²⁷⁻²⁹.

Alternatively, if pinocytosis of serum proteins is actually the rate-limiting step in their degradation, the structural features of the serum proteins which determine their rates of pinocytosis must correlate with their protease sensitivity (Fig. 1) and their isoelectric points (Fig. 2). For example, proteins that are more sensitive to proteases or more acidic may tend to denature more easily^{5,6}, and denaturation can promote pinocytosis^{9,30}. Whatever the mechanism underlying the correlations with charge and proteolytic susceptibility, the fact that they apply to both intracellular and extracellular proteins suggests that degradation of these two types of proteins involves similar principles and may even share common mechanisms.

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Accessibility of DNA in condensed chromatin to nuclease digestion

RECENT evidence indicates that a large portion of the DNA of higher organisms is organised in compact nucleoprotein structures. Initial studies on nuclease digestion of chromatin showed that about half the nuclear DNA is present in the form of small resistant chromatin fragments¹⁻³. Subsequent studies have shown that the sites of nuclease digestion are regularly spaced, and that, at the limit of digestion, the majority of the DNA that remains is in the form of relatively homogeneous small fragments, which vary from about 120 to 200 nucleotide pairs in length⁴⁻⁷. This concept of small repeating resistant structures in chromatin is further supported by electron microscope studies on chromatin fibres spread from hypotonically treated nuclei⁸ and from isolated chromatin⁹.

Here we examine whether two types of 'condensed' chromatin—mitotic chromosomes and the fraction of interphase chromatin that contains satellite DNA—have the same subunit structure as 'non-condensed' chromatin. The experiments were performed on cells of *Dipodomys ordii* (kangaroo rat) because (1) mitotic cells can be shaken off monolayer cultures selectively to yield pure mitotic cell preparations and (2) this species contains 52% of its DNA in the form of satellite components¹⁰ which are associated with a dense interphase chromatin fraction¹¹.

The kinetics of staphylococcal nuclease digestion of DNA in interphase nuclei or mitotic chromosomes to acid-soluble material are essentially identical. At an enzyme concentration of 30 units ml⁻¹ there is a rapid phase of digestion such that 65% of the DNA remains acid insoluble after 15 min, followed by a slower linear digestion until 50% remains acid insoluble after 1 h. Accessibility of DNA to the enzyme is similar in both chromatin states.

Another test for similarity in the sub-structure of interphase nuclei and mitotic chromosomes is to observe the size distribution of DNA fragments generated at various times during nuclease digestion of the chromatin. Cells were uniformly labelled with ³H-thymidine for two cell generations. ³H-labelled chromosomes and ³H-labelled interphase nuclei were prepared from these cultures and separately mixed with unlabelled interphase nuclei in the ratio of 1:10 in DNA amount. DNA extracted from partial nuclease digests was analysed on 1.5% Agarose gels which were photographed after staining with ethidium bromide. The gels were subsequently processed for fluorography, and the sizes of unlabelled DNA fragments detected by ethidium bromide staining were compared with the sizes of ³H-labelled DNA fragments detected by fluorography (Fig. 1).

At all stages of nuclease digestion of the chromatin mixtures we could detect no differences in the size of the DNA fragments from single or multiple subunits derived from unlabelled interphase chromatin (Fig. 1a and c), ³H-labelled interphase chromatin (Fig. 1b), or mitotic

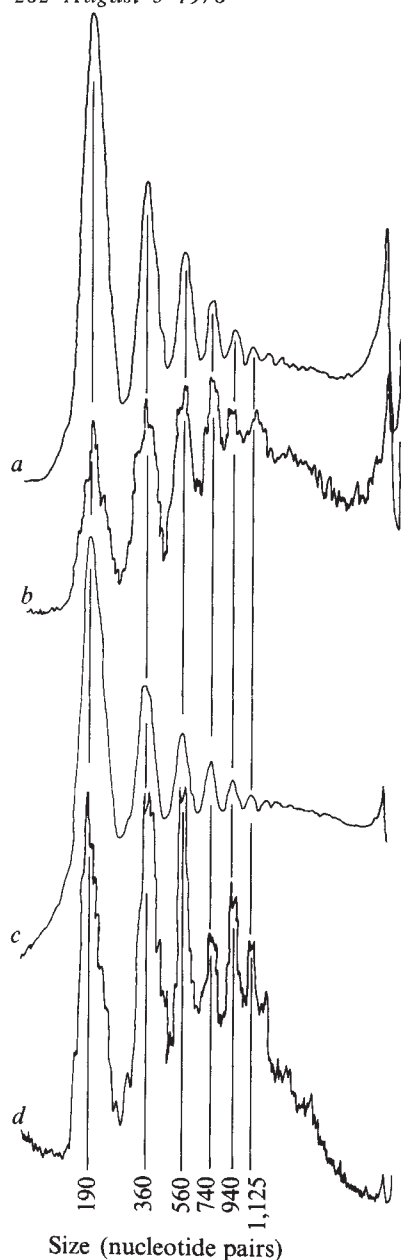


Fig. 1 Agarose gel and fluorogram analysis of DNA from interphase and mitotic chromatin digested with staphylococcal nuclease. Mitotic and interphase cells of *D. ordii*, labelled for 60 h with 0.2 μCi ^3H -thymidine (54 Ci mM^{-1} , Amersham) in F-12 medium lacking unlabelled thymidine, were collected separately as described elsewhere¹³. Equal quantities of each ^3H -labelled sample were mixed with 10 times the number of unlabelled cells. The nuclei and chromosomes were prepared by one of a number of methods^{3,14,15}, and resuspended in 20 mM Tris, 1 mM CaCl_2 , 1 mM MgCl_2 , pH 7.5 at a concentration equivalent to 200 μg total DNA ml^{-1} . Staphylococcal nuclease was added at 30 units ml^{-1} . At various intervals after the start of digestion, samples were removed and digestion stopped by addition of 2 ml 50 mM sodium phosphate buffer, pH 6.8, containing 5 mM EDTA. Sodium dodecylsarcosinate and NaCl were added to final concentrations of 0.5% and 1 M respectively, and the lysate was extracted twice with water-saturated phenol. DNA was precipitated by the addition of two volumes of absolute ethanol followed by storage overnight at -20°C . DNA precipitates were collected by centrifugation, redissolved in 10 mM Tris, 10 mM EDTA, pH 8.0, and electrophoresed in 1.5% Agarose slab gels (18 cm $\times$ 18 cm $\times$ 5 mm) for 16 h at 10 mA at room temperature. The electrophoresis buffer was 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 8.2. Gels were stained with 0.5 μg ml^{-1} ethidium bromide dissolved in electrophoresis buffer, and the fluorescence photographed under light at 254 nm. Photographed gels were processed for fluorography essentially as described by Bonner and Laskey¹⁶ except that ethoxyethanol was used as the solvent in place of dimethyl sulphoxide. Fluorograms were exposed on presensitised Kodak RP-Royal Xomat film at -75°C and developed in Kodak D-19 developer. Photographic negatives of the stained gels and the fluorograms were

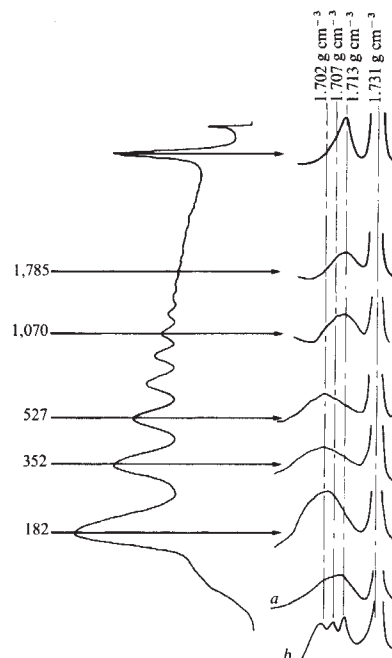


Fig. 2 Caesium chloride neutral density gradient analysis of DNA of different fragment sizes following partial digestion of nuclei by nuclease. Nuclei were isolated and digested with staphylococcal nuclease and then several slab gels were run as described in Fig. 1. The individual DNA bands were cut out, and the DNA was recovered by crushing the gel through a 24-gauge hypodermic needle and centrifuging the crushed gel at 200,000g for 30 min. The supernatant was removed, solid caesium chloride added to a density of 1.70 g cm^{-3} and ethidium bromide extracted with *n*-propanol. After removal of ethidium bromide, the density was adjusted to 1.710 g cm^{-3} and *Micrococcus lysodeikticus* DNA was added as a density marker. Centrifugation was at 44,700 r.p.m. at 25°C for 24–48 h in the 6-place analytical rotor in an MSE Centriscan. Left, Densitometer tracing of a photographic negative of DNA from a partial digest separated on 1.5% Agarose gel as described in Fig. 1. Right, Neutral caesium chloride banding profiles of DNA isolated from the various regions of the gel as indicated. The buoyant density profiles of *a*, total DNA after partial digestion and *b*, total undigested DNA, are shown at the bottom. The size of the DNA fragments (in base pairs, see Fig. 1) is indicated on the left.

chromatin (Fig. 1d). The distribution of the majority of nuclease-sensitive sites is thus the same in both mitotic and interphase chromatin.

We have measured the relative rates of digestion of the fractions containing satellite and non-satellite DNA in interphase chromatin. The satellite DNA component is less rapidly digested by nuclease than chromatin containing non-satellite DNA. We have shown this by measuring the amounts of satellite DNAs present in DNA fragments of different size at various stages of digestion of interphase chromatin. The DNA fragments were recovered from Agarose gels and analysed by analytical caesium chloride density centrifugation or thermal denaturation in 0.1 $\times$ SSC. The three satellite DNAs of *D. ordii* band on the heavy side of the main band DNA. HS- α and HS- β satellite DNAs

scanned in a Joyce Loebel densitometer. The tracings shown are both of chromatin digested for 15 min (20% of the DNA rendered acid soluble). *a*, Ethidium bromide fluorescence distribution; unlabelled plus ^3H -labelled interphase chromatin; *b*, distribution of ^3H -labelled DNA fragments from interphase chromatin; *c*, ethidium bromide fluorescence distribution; unlabelled interphase chromatin plus ^3H -labelled mitotic chromatin; *d*, distribution of ^3H -labelled DNA fragments from mitotic chromatin. The size of the DNA fragments (in base pairs) is indicated at the bottom of the figure. These values were obtained by comparison with DNA fragments produced by *Eco*RII restriction enzyme digestion of mouse satellite DNA¹⁷ and *Hind*III restriction enzyme digestion of SV40 DNA¹⁸.

have a density of 1.713 g cm^{-3} and MS satellite DNA has a density of 1.707 g cm^{-3} (ref. 12). The HS- β satellite has a G+C content of 66%, and can be distinguished clearly as a separate component in a thermal melting profile. Buoyant density analysis has the advantage that it can identify all three major satellite components, but the disadvantage of giving poor resolution at the molecular sizes of the smaller DNA fragments. Thermal denaturation, on the other hand, can reliably distinguish HS- β satellite DNA component in large and small DNA fragments, but can only detect about 20% of the total satellite DNA.

Buoyant density analysis of DNA of different fragment sizes from partially digested chromatin (Fig. 2) shows that the DNA remaining as high molecular weight structures—larger than six times the single subunit size—is almost entirely satellite DNA. DNA fragments of single subunit size, or low multiples thereof, have broad banding profiles,

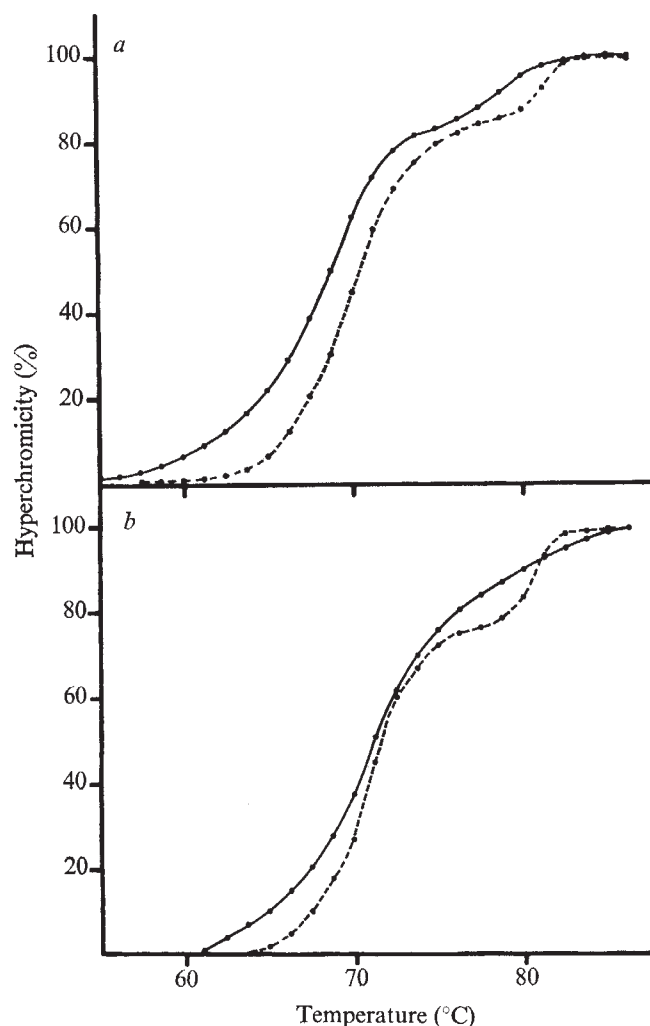


Fig. 3 Thermal denaturation profiles of *a*, total undigested DNA (—) and single subunit sized DNA after complete nuclease digestion (---). *b*, Single subunit sized DNA (—) from partially digested (20% acid soluble) chromatin and high molecular weight DNA (---) fragments of partially digested (20% acid soluble) chromatin. Total undigested DNA was isolated from nuclei by the method of Marmur¹⁹ with slight modifications. DNA fragments from digested chromatin were recovered from the supernatants of crushed gels (see Fig. 1) by adding NaCl to a final concentration of 1 M, extracting twice with 2 volumes of water-saturated phenol, and precipitating the DNA by addition of 2 volumes of ethanol followed by storage overnight at -20°C . The precipitates were collected by centrifugation, dissolved in $1/100 \times \text{SSC}$ and dialysed exhaustively against $1/10 \text{ SSC}$. All samples were thermally denatured in $1/10 \text{ SSC}$ in a Unicam SP800 spectrophotometer. (SSC is 0.15 M sodium chloride, 0.015 M sodium citrate.)

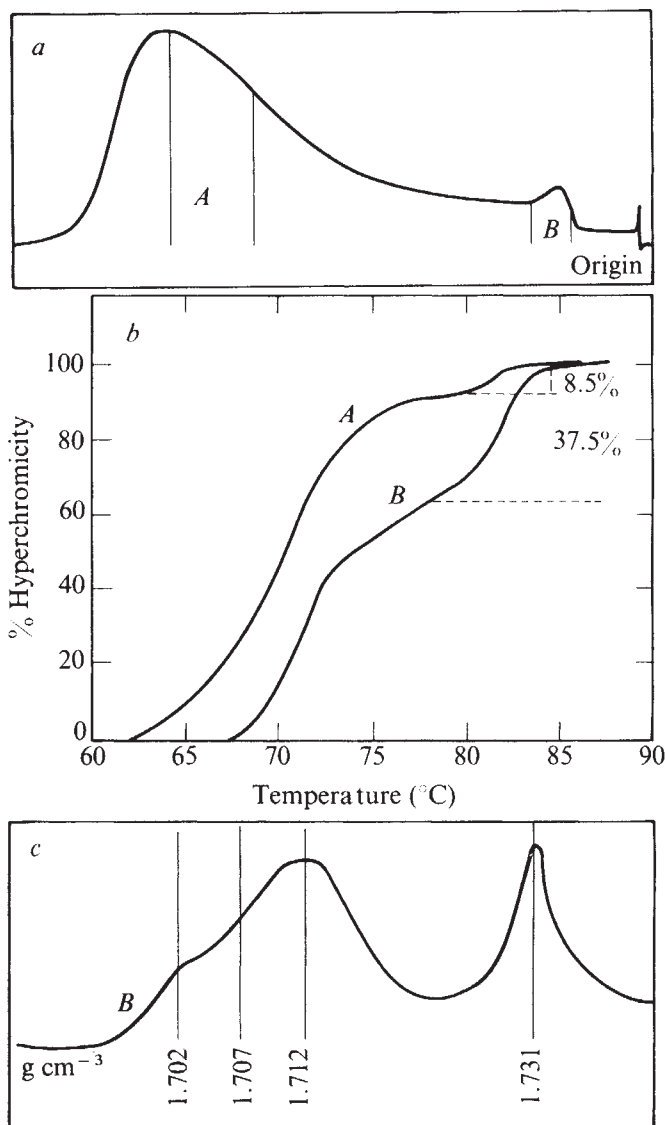


Fig. 4 Whole DNA from cells of *D. ordii* was isolated by the method of Marmur¹⁹ with slight modification, and digested with staphylococcal nuclease to an extent comparable with the partial chromatin digestions of Figs 1 and 2. The DNA was re-extracted and electrophoresed in a 1.5% Agarose slab gel as in Fig. 1. *a*, The fluorescence pattern of ethidium bromide was photographed and scanned. Migration was from right to left. Zone A DNA migrated a distance approximately equal to the 180-base pair subunit of chromatin and zone B DNA was of high molecular weight near the exclusion limit of the gel. These zones were pooled from several gels and the DNA fractions were recovered as described in Figs 2 and 3. *b*, Aliquots of DNA from zones A and B were dialysed against $1/10 \text{ SSC}$ and denatured thermally. *c*, DNA of high molecular weight from zone B was centrifuged to equilibrium in neutral CsCl as described in Fig. 2.

but with a peak density characteristic of main band DNA. The enrichment of satellite DNA in high molecular weight DNA fragments is unambiguous, but the depletion of satellite DNA in low molecular weight pieces is less convincing, owing to the diffuse banding profiles.

This ambiguity is overcome by thermal denaturation. When chromatin was digested for 1 h (as in Fig. 1) the 50% of DNA that remained acid insoluble was entirely at the size of single subunits. The melting profile of this DNA was similar to that of total undigested DNA (Fig. 3a). Although the T_m of the single subunit-sized DNA is lower because of its decreased molecular weight²⁰, the second transition (HS- β satellite DNA) is about 15% of the total hyperchromicity in both samples. Thus, at this stage of digestion to single subunit size, the distribution of satellite

and non-satellite DNAs is the same as in undigested chromatin. In a partial digest of chromatin, however, (Fig. 3b) DNA of single subunit size is considerably depleted HS- β DNA, whereas DNA remaining at high molecular weight is enriched in HS- β to a degree consistent with the sample containing only the total satellite mixture.

We are grateful to a reviewer for the suggestion that the apparent resistance of satellite DNA-containing chromatin during brief digestion could be due to the known preference of the nuclease for AT-rich DNA²¹. As a result, free DNA isolated from cells of *D. ordii* was digested to various extents in the same conditions as for chromatin digestion. Similar to the partial chromatin digestion (Fig. 3), most of the DNA fragments formed a broad band in 1.5% Agarose gel, the size of the single subunit of chromatin and smaller, whereas a small amount of DNA was almost excluded from the gel (Fig. 4a). Zones at approximately 180-base pair size and of high molecular weight were extracted and the recovered DNA fractions were centrifuged in a CsCl gradient and melted. The density gradient pattern of the high molecular weight DNA (Fig. 4c) corresponded with the 1,070-base pair pattern of Fig. 2, thus showing enrichment in satellites and depletion of main band DNA. Thermal denaturation (Fig. 4b) revealed that the second transition (representing HS- β satellite) was smaller than that of whole DNA for the low molecular weight fraction and very much larger for the high molecular weight fraction.

The results with partial digestion of free DNA are very similar to those of chromatin. The apparent resistance of chromatin containing satellite DNA to nuclease digestion may be due to the base preference of the enzyme rather than to a difference between extended and condensed chromatin in accessibility of DNA to the enzyme. Other factors may, however, be involved since only HS- β satellite is substantially richer in GC content than main band DNA, yet all three satellites seem to behave similarly towards nuclease digestion. This question is receiving further study.

From our results it can be concluded that, firstly, the structures of extended and condensed chromatin in the forms of mitotic chromosomes or satellite DNA-containing interphase chromatin do not differ significantly in respect to accessibility to digestion by staphylococcal nuclease and secondly, the size and distribution of nuclease-resistant chromatin subunits are similar in extended and condensed chromatin. These findings support models of chromatin structure which place the DNA moiety in an exposed position outside the subunits²²⁻²⁵. A condensed state of chromatin does not arise therefore from greater coverage by proteins in a manner that obstructs the access of nuclease to the DNA.

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Amphipathic enzyme-polymer conjugates

IMMOBILISATION of enzymes on solid supports to give reusable and stabilised catalysts has been studied extensively¹ but the concept of chemically modifying a water-soluble enzyme to enable its activity to be utilised in the form of a water-immiscible liquid phase has received little attention. Potential advantages of liquid-phase immobilisation include ease of continuous operation, freedom from the effects of solid particle breakdown and the possibility of enhanced activity against water-insoluble liquid substrates. Although the effects of detergent-protein association on protein extractability into *iso*-octane have been studied² and considerable effort has been expended on the liquid membrane entrapment concept^{3,4}, another possible type of liquid-phase immobilised enzyme system seems to have been neglected. The latter comprises droplets of a water-immiscible liquid bearing a surface layer of modified enzyme. We have found that some enzymes may be converted into surface-active amphipathic conjugates by covalent coupling to certain types of polymeric detergents (polysoaps) and that the resulting conjugates may bind sufficiently strongly to dispersed water-immiscible liquids to give an enzymatically active emulsion that can be handled as a quasi-liquid phase. The amphipathic enzyme conjugates are analogous to certain membrane-bound proteins such as cytochrome *b₅*, which have been shown to comprise a hydrophilic protein core anchored to a lipid bilayer by a hydrophobic polypeptide tail^{5,6}.

A particularly simple and convenient route to conjugates of proteins with random copolymer amphipaths is shown in Fig. 1.

A linear hydrophilic polymer such as the non-cross-linked types of ethylene-maleic anhydride copolymers previously used for enzyme immobilisation^{7,8} or the maleic anhydride-vinyl methyl ether copolymer Gantrez AN⁹ can be modified with hydrophobic nucleophiles (for example, RNH₂) by partial substitution of the anhydride functions. The remaining anhydride units may then be used to couple the polymer to a protein.

The modification reaction is conveniently carried out in *NN*-dimethylformamide in the presence of an organic base and the reaction mixture can be added directly to a solution of the enzyme with homogenisation. Alternatively, the modified polymer can be hydrolysed and the protein coupled to it using established carboxyl group activation techniques such as conversion to the acid azide¹⁰ and the use of water-soluble carbodiimides¹¹ or Woodward's reagent K (ref. 12). These general methods enable control of net hydrophobicity, degree of enzyme substitution and, since cross linking by the protein can be minimal, of conjugate molecular weight. There is scope for introduction of spacer groups, different enzymes on the same polymer and other refinements.

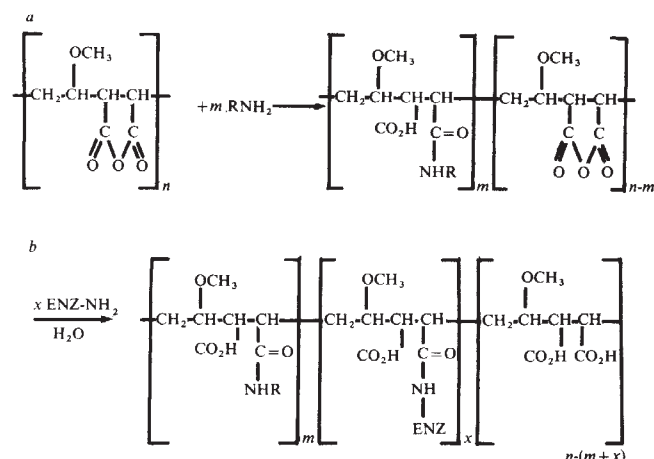


Fig. 1 Route to conjugates of proteins with a random copolymer amphipath.

Using the technique described, penicillin acylase and other enzymes have been conjugated with good recovery of enzyme activity. For example: a polymer solution prepared from Gantrez AN119 (0.5 g) and *n*-octadecylamine (0.28 g) in *NN*-dimethylformamide (2.5 ml) and pyridine (0.1 ml) was homogenised with *Escherichia coli* penicillin acylase (175 mg partially purified enzyme) in water (30 ml). After reaction at 4 °C overnight, sodium chloride (5.8 g) was added to precipitate the amphipathic solute, and the polymer conjugate isolated by centrifugation at 25,000*g* at 0 °C for 1 h. A gel (4.0 g wet weight) of high specific activity resulted, and recovery of enzyme activity was 70%. Table 1 lists further conjugates of the same type. The physical form of these preparations varies from highly aggregated solids that sediment rapidly to materials that are soluble in low ionic strength aqueous media according to the extent of substitution by hydrophobic groups in the polymer or protein in the final conjugate. Some conjugate preparations may be fractionated by salt precipitation into materials of varying net hydrophobicity. Enzyme lability does not usually increase on conjugation, indicating that linkage to a polymeric detergent does not necessarily destabilise the catalytically active conformation of a protein.

Homogenisation of such conjugates with buffer and liquids such as *n*-decane, *n*-decanol and glycerol trioleate results in emulsions which can readily be separated by flotation or gentle centrifugation into an aqueous phase and a droplet phase with which the enzyme activity is associated. For example: homogenisation of the penicillin acylase conjugate described in the text above, with *n*-decanol in a wet gel-solvent weight ratio of 1:5 resulted in 90% of the enzyme activity being associated with the droplet phase on separation. The unconjugated enzyme does not bind significantly to solvent droplets formed using the Gantrez-based polysoaps. Some of the enzymatically active emulsions are quite stable: the droplet phase described above retained more than 40% of the initial enzyme activity after ten flotation-reuse cycles (batch cleavage of 5% w/v benzylpenicillin). Binding of the conjugates to dispersed phospholipid-cholesterol mixtures and whole cells has been observed. Photomicrography of solvent dispersions produced with penicillin acylase and other conjugates shows that solid gel particles are not visible at the droplet surfaces; and it is likely that the conjugate is distributed largely as a monolayer. If bifunctional spacer groups or enzyme coupling agents such as glutaraldehyde are used in the preparation of conjugates, the resulting cross-linked gels form visible aggregates which, when dispersed with a solvent, adhere to the surface of the droplets. Such a three-phase sludge can, nevertheless, be handled as a discrete enzymatically active layer.

Table 1 Amphipathic enzyme conjugates*

Enzyme	Hydrophobic group $\text{CH}_3(\text{CH}_2)_y\text{NH}-$	$m/n^\dagger$	Method of coupling polymer to protein	% Recovered enzyme activity
<i>E. coli</i> penicillin acylase	$y = 11$	0.33	Direct reaction with anhydride polymer [¶]	80
<i>E. coli</i> penicillin acylase	17	0.33	Conversion to half hydrazide polymer and activation to polymer azide	32
Bovine trypsin	9	0.30	Direct reaction with anhydride polymer ^{**}	24
Bovine trypsin	17	0.33 [§]	Carbodiimide activation of hydrolysed polymer carboxyl groups ^{††}	~ 5

*Based on Gantrez AN119-substituted polymers. *m* and *n* refer to Fig. 1.

[†]Stoichiometry in polymer modification reaction, $n \approx 1,500$.

[‡]Based on activity in original coupling mixture.

[§] $n \approx 5,000$.

[¶]Ether-precipitated polymer (0.25 g) dissolved in *NN*-dimethylformamide (1 ml), homogenised with acylase (100 mg) in 0.1 M sodium phosphate, pH 7.0 (14 ml), reacted for 16 h at 4 °C, and conjugate gel isolated by precipitation with ammonium sulphate (70% saturation).

^{||}Hydrazide polymer prepared from anhydride polymer (21 μmol) and hydrazine hydrate (0.2 mol) in *NN*-dimethylformamide (50 ml). Polymer isolated by precipitation from 1.4 M aqueous NaCl (500 ml). Wet polymer (5 g) activated with NaNO₂ (0.5 g) in 2 N HCl (50 ml) and reacted with acylase (55 mg) in water (100 ml). Gel isolated by centrifugation.

^{**}Polymer reaction mixture from Gantrez AN119 (1.0 g), *n*-decylamine (0.3 g) in *NN*-dimethylformamide (6 ml) added to trypsin (0.3 g) in 0.1 M sodium phosphate, pH 7.0 (60 ml). Titrated with 2 N NaOH until base uptake complete and gel isolated by successive precipitations with 1 M NaCl and 70% saturated ammonium sulphate.

^{††}Hydrolysed polymer gel (15 g wet weight) reacted with 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulphonate (0.5 g) in 0.1 M sodium phosphate, pH 4.7 (25 ml) for 5 min at room temperature; then treated with bovine trypsin (75 mg) at pH 5.9 and room temperature for 3 h. Conjugate gel isolated by centrifugation.

The amphipathic conjugation technique is a novel method of modifying proteins and has a large number of possible applications. In the field of biotechnology it provides a means of immobilising enzymes on solvent droplets, or, in the case of water-soluble conjugates, effecting the extraction of enzyme activity from an aqueous phase. It could also be used for locating well-characterised enzymes on natural or model biological membranes. Further details of the techniques, for which patents are pending, will be presented elsewhere.

I thank Dr J. Green for discussions and Mr I. Dodd for assistance.

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reviews

Nutrition and the developing brain

John Dobbing

Malnutrition and Brain Development. By Myron Winick. Pp. xv+169. (Oxford University: Oxford and New York, April 1976.) £5.

Nutrition and the Developing Nervous System. By Philip R. Dodge, Arthur L. Prenskey and Ralph D. Feigin. Pp. xvi+538. (Mosby: Saint Louis; Kimp-ton: London, January 1976.) £27.25.

THE suspicion is very strong that malnutrition during fetal and early neonatal life may lead to an irrecoverable diminution of intellect: and since the majority of the pregnant mothers and children in the world are underfed, this adds up to an assault on one of man's most distinguishing features by environmental circumstances which are theoretically within his control. The problem is proving it.

Human achievement is difficult to measure in any significant terms. Human growth from babyhood to adulthood is very slow, and nothing short of a longitudinal prospective study over many years is going to be satisfactory in communities where a reliable retrospective history of fetal and baby life is always especially difficult. Environmental contributions to the shaping of personal development (which are of course so numerous and so powerful compared with any genetic contribution) are nevertheless very difficult to enumerate and evaluate separately, especially as they are interacting as well as additive.

One mechanism amongst many by which early malnutrition may reduce eventual performance, may be by way of permanent alterations to the structure and some of the metabolic biochemistry of the physical brain, and we are now beginning to understand a great deal about this. Unfortunately the less well nourished members of any infant community who will probably suffer from this physical neuropathology are usually the most impoverished in every other facet of their environment, both physical and 'emotional'; and so it is exceedingly difficult to know whether the newly discovered growth pathology of the brain matters, and if so how (relatively) much?

Both these books are predominantly about the physical effects of nutrition on the developing brain, and as such inevitably only consider that one aspect

of the huge problem in any detail. Dodge's book is a compendious and suitably critical account for the serious student and is likely to have few competitors for a good time to come.

If Winick's much smaller book had been simplified in the sense of being a reliable introduction for the less professionally interested it could have been very useful for a wider readership. Unfortunately it is not. I think Winick confuses the main issues of vulnerable periods in developing brain and the relative importance of fetal and post-natal brain growth, to the point of being potentially misleading to nutritionist and politician alike; and in spite of its attractive appearance I am worried by it.

Dodge, painstakingly and with abundant references, reasons his way through a morass of much incompletely collected and questionably argued literature with remarkable astuteness; although he and his colleagues have occasionally tended to catalogue the information rather than evaluate it.

Both books in their way cover the

elements of normal brain growth; and since the brain is an organ of the body like any other, both have paid attention to the very relevant effects of nutrition on somatic growth.

Dodge, as a clinical paediatric neurologist, adds some valuable shorter sections on minerals, vitamins and diseases of amino acid metabolism with implications for the brain. Neither book seems to have heard of our own finding that the period of vulnerability of the human brain is almost certainly predominantly postnatal, an important fact for practical social and political policy; or perhaps they do not agree. At all events they might have said so. Dodge discusses the rest of our work in Manchester very fully and appreciatively, but I do not think my very strong preference for his version over Winick's is entirely due to that. □

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Aspects of polymeric carbons

Polymeric Carbons: Carbon Fibre, Glass and Char. By G. M. Jenkins and K. Kawamura. Pp. vi+178. (Cambridge University: Cambridge and London, May 1976.) £8.50.

THE decomposition of resins at temperatures up to 2,500 °C gives rise to graphite in the form of small and highly distorted crystals. As every Materials Scientist knows such distortions can confer strength well above that of more perfect graphite; the resultant materials are very useful but very difficult to characterise. Vitreous carbon is used for crucibles and in some biomedical applications where high strength, chemical inertness or high temperature resistance are needed; whereas carbon fibres are used in a wide range of composites.

This book covers the production, properties and structure of these polymeric carbons and puts them in the context of other forms of carbon. The style is easy to read and the treatment is mainly factual, the emphasis being on what happens rather than why. The 200 references cover

the scientific and patent literature up to 1973. A lot of attention is given to the author's own work on pyrolysed phenolic resins, which lends the book coherence.

As a text it has its limitations; there is not enough theoretical explanation and the treatment of chemistry is sloppy. For instance, the statement that "Commercial PAN is atactic and so contains two-dimensional order only" is simplistic, considering the importance of the resultant carbon structure on properties. It is also regrettable that there is no discussion of surface structure and properties when these are so important in practice.

In spite of this, for people in research or development using polymeric carbons, this monograph will be a good introduction and guide to the literature. Here, it fills a real lack.

Paul Calvert

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Beauty of original experiments

Landmark Experiments in Twentieth Century Physics. By George L. Trigg. Pp. x+303. (Crane Russak: New York; Edward Arnold: London, February 1976.) £11.50.

It is a sad fact, but unfortunately a true one, that most physicists' knowledge of the fundamental papers of their subject can only be described as fragmentary, even if one is feeling charitable. How many of us have in fact read the paper by Michelson and Morley, or the verification using α -particle scattering of the Rutherford nuclear atom by Geiger and Marsden, or nearer at hand the papers on the de Broglie wavelength of the electron by Davisson and Germer or G. P. Thomson.

We all know of the existence of these experiments and what they prove but our knowledge is second-hand, acquired from the writings of others. It is perhaps not as bad as reading Shakespeare through the eyes of another person, since aesthetic appreciation doesn't enter. If as sometimes happens for a colloquium or lecture course however, we have to examine a bit of fundamental experimentation in detail it is surprising how much rationalisation and perhaps even falsification enters the conventional picture.

This is not deliberate misrepresentation, but it is just that the removal of an awkward corner or fact here and there facilitates understanding and is thus tacitly accepted. For example, one often finds the Michelson-Morley experiment discussed in terms of the time of arrival of pulses of light going down the separate paths. No doubt if the experiment were done this way the result would still be as given and the consequences would be identical; but it is a pity to depart from complete accuracy.

The whole situation stems from the expansion in physics over the past few decades, the need to keep university courses to a sensible length, and the reluctance of physicists to split their subject into more manageable parts. Tiresome examiners at the end of the three undergraduate years demand that we show some powers as practising physicists able to marshal our equations and solve our problems. They are not so concerned as a rule with the way the subject has developed to its present state. Schrödinger's equation, or operators in quantum mechanics, or matrix algebra take precedence over Davisson and Germer, and de Broglie. In this of course they are right, but something important is being lost.

This is true also of books, in which as a particular subject matures so the formal structure grows and the experimental details recede. The present book, however, is of a different kind and attempts to redress the balance. The author takes a list of important twentieth-century experiments, first puts them briefly in their context, and then goes on to describe what was really done in great detail. To do this he uses long sections taken from the original papers which he joins and makes coherent by a minimum of connecting explanation. He thus maintains the authentic flavour of the subject at the time of the experiment; and much of the work is described in the original author's phraseology.

The list of experiments discussed is reasonably well chosen. It begins with the discovery of the wave nature of X-rays using diffraction from crystals, of isotopes, of the concept of atomic number and of superconductivity. During this early period of the century many other possible subjects could of course be chosen but the ones used are not at all unreasonable. It may be that if the book is successful the author might return to this early era and write a second volume on some of the experiments he has omitted.

After this early period, and in fact, coming within the reviewer's memory, the list becomes more restricted and progressively less open to variation. From the early 1930s to the present day the complete list of topics covers

the fluid properties of liquid helium below the λ point, the use of molecular beams for the determination of magnetic moments, the Lamb-Retherford effect, the anomalous g factor for the electron, the transistor, the non-conservation of parity for weak interactions, the Mössbauer effect, the experimental verification of the existence of the neutrino, the maser and laser, tunneling and superconductivity, the Ω^- meson and the microwave demonstration of the 3K intergalactic radiation temperature.

In spite of its intrinsic interest the book is not easy to read. The subjects cover quite a good cross section of contemporary physics and the use of extracts from the original papers sets a level of difficulty which cannot be entirely overcome by the explanatory connecting parts. This, however, cannot be helped. A student of scientific history or of scientific logic or of experimental technique or anyone just interested in how things really happened could not do better than begin with this book. The reader will get in a few hours an insight which has obviously taken the author a long time and much labour to achieve, and will be left with a feeling that at least some experiments in physics have a beauty which is more akin to art than science.

R. Latham

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Nucleon-nucleon forces

The Nucleon-Nucleon Interaction. By G. E. Brown and A. D. Jackson. Pp. viii+242. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1976.) n.p.

WHEN students start research on a particular topic in theoretical physics their first problem is to learn all the essentials of previous work in a finite time. This nearly impossible task is helped by providing them with courses of lectures in which early developments are outlined and more recent work is spelled out in sufficient detail for the student to proceed immediately to the frontiers of the subject equipped to make further progress. G. E. Brown and A. D. Jackson have given such courses in Copenhagen which form the basis of their book. The first four chapters give a simple outline of the well-established theory of nucleon interactions at low energies. In chapters 5 and 6 the reader is introduced to techniques in scattering theory, the

use of relativistic equations and form factors; a description is also included of a more specialised technique, the eikonalisation of soft vector bosons, which is used by the Copenhagen group to provide convergence.

The final four chapters (half the book) are devoted to a detailed and explicit account of the steps required to derive realistic internucleon potentials and their relativistic corrections from one- and two-boson exchanges. The intimate interweaving of the various pion-nucleon and nucleon-nucleon amplitudes is explicitly demonstrated and a clear picture emerges of the physical principles underlying the calculations. This book will be essential reading and remain a basic reference for all those working on nucleon-nucleon forces, as well as being an introduction to the subject for the uninitiated.

Leonardo Castillejo

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Living history of science

Pioneers in Neuroendocrinology. (Perspectives in Neuroendocrine Research, Vol. 1.) Edited by Joseph Meites, Bernard T. Donovan and Samuel M. McCann. Pp. viii+327. (Plenum: New York and London, 1975.) \$27.

THE majority of the scientists that have ever lived are alive at the present time. Thus it is possible for followers of the ever-growing subject of the History of Science to actually discourse with the people who made this history: they have the opportunity to examine at first hand the politics, philosophy, and circumstances that resulted in the scientific progress that has been made.

The subject of Neuroendocrinology—the study of the effects of the nervous system on the release of hormones, as well as the study of the effects of hormones on the nervous system—is a particularly young discipline, having only evolved since the period between the two world wars. Thus Joe Meites, Bernard Donovan and 'Don' McCann (themselves pioneers in this field) have been able to seize successfully the opportunity to call on 21 neuroendocrine pioneers "to write a personal, and even idiosyncratic, account" of the background behind the major contributions that each felt he or she had made to this subject. The editors have indeed extracted idiosyncratic accounts from their authors, and each chapter can very much be considered to reflect the character of the respective contributor. Whereas some authors, therefore, have used their publications as mileposts in the passage of time, others have given only a very technical and clinical survey of their research careers, without reference to other events occurring at the time, either in the scientific world, or in the world at large (such reference, if included, being of the greatest value and interest to the historian and to the lay-reader).

There are three chapters that I should particularly like to single out—those by the late Hans Heller, by Dora Jacobsohn, and by Dorothy Price—in that not only do they excel in the good qualities described above, but they also reflect the charm and humility of their respective authors.

It is regrettable that in the present volume two of the chapters are of a similar nature to the respective chapters in a comparable volume, *The Neurosciences: Paths of Discovery* (MIT Press, Boston). This book has, however, already met sufficient acclaim in America that the editors are presently commissioning writers for a second volume. That there should be so many pioneers available, and willing, to write



Hunting Nubian ibex (*Capra nubiana*) in the Red Sea Hills of the Sudan. Nubian ibex have long, slender, knotted horns, gracefully curved. Among males, 42-inch trophies are not uncommon, and the new world record (shown above) measures 54.5 inches. Taken from *African Hunter*. By James Mellon. Pp. xx+522. (Casell: London, June, 1976.) £15.00.

presumably indicates that each and every scientist is a pioneer in his or her own way: the willingness, as pointed out by M. C. Shelesnyak, derives, at least in part, from the scientist "seeking self-satisfaction and nurturing (his) ego".

Although I would recommend that anyone involved in endocrine or physiological research should read this book, since they will do so with interest, I feel that the price is such that few will be able to afford to buy it. I fear, too, that this book is not sufficiently 'applied' for most science libraries to purchase, unless, that is, they have a shelf principally intended for reference by after-dinner speakers.

Barend ter Haar

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Climate and man

Climate and the Environment: The Atmospheric Impact on Man. (Environmental Studies.) By John F. Griffiths. Pp. vii+148. (Elek: London, May 1976.) £2.95.

INTENDED for "students of geography and environmental studies at undergraduate and pre-university level" this book is a useful quick guide to the relationship between the workings of the atmosphere and the activities of man, without anything like the in-depth treatment of M. I. Budyko's classic work *Climate and Life* (Academic: New York and London,

1974; for review, see *Nature*, 251, 362). The comparison is a natural one, since Griffiths approaches his subject through the energy budget-heat balance approach, drawing to some extent on the work of the Voeikov Geophysical Observatory, where Budyko was until recently Director. He also draws on the work of many other climatologists, as is usual in a book of this kind, but with comprehensive references to the original sources at the end of each chapter—a rarer, but welcome, practice for a text at this level.

There are many clear and useful illustrations, again culled from a variety of published works, and the text is well suited to the intended readership, with some of the mathematical details contained in appendices, again conveniently at the end of the appropriate chapters. The success of the author in reaching his planned market does, however, limit the usefulness of the book to anyone with a more advanced knowledge of the physical sciences who might have become interested in the subject through the continuing debate about the impact of climatic change on man, and the impact of man on climatic change. In the specified teaching context this book is likely to prove invaluable and is strongly recommended; at the next step up the academic ladder, however, Budyko's book remains the essential introduction to the subject. **John Gribbin**

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obituary

Alexander Naumovich Frumkin the father of modern electrochemistry died in Tula (200 km from Moscow) after a heart attack on May 27, 1976.

He was born in 1895 in Kishinev, capital of Moldavia (now the Moldavian Soviet Socialist Republic), and attended the St Paul's Real School in Odessa, 150 km from his birthplace. On leaving school in 1912, he studied in Strasbourg and Bern but returned to graduate in the physico-mathematical faculty of the Novorussian University at Odessa, and become a staff member there. During this period he laid the foundations of the electrochemical studies which formed the mainstream of his work. His thesis, published in 1919 (he never in fact received a doctorate, as such distinctions were abolished in the early days of revolutionary Russia) besides giving a masterly survey of the current views on these problems, contained several notable original contributions (among which, many of the major themes of Frumkin's later work may be seen to have their origin): a careful experimental test of the basic equations of electrocapillarity, the identity of the point of zero charge determined by several methods, how Gibbs' equation may be used to derive surface excesses from electrocapillary curves, the nature of the potential-dependence of the adsorption isotherm, the lack of a simple relation between the double-layer capacity and the bulk dielectric constant, and finally the origin of the e.m.f. of a galvanic cell (one of the basic problems of electrochemistry).

In 1922 he moved to the Karpov Institute of Physical Chemistry in Moscow, formed to investigate the

fundamental physico-chemical basis of industrial processes. Here he extended his interests to the gas-liquid interface, to the adsorption of electrolytes on activated charcoal and platinum black and to the problems of wetting and contact angles. He made pioneer measurements of the Volta potentials of solutions and showed how they depended on molecular orientation at the surface. His outstanding research was recognised by his election to the Academy of Sciences of the USSR.

His work in the decade after 1930 was even more important for electrochemistry. His first achievement was the marriage of surface chemistry and electrode reactions, in a paper which brilliantly used some earlier polarographic studies to give a rational theory of the salt effect on electrode reactions. The second major success was the first reliable direct measurement of the double-layer capacity and the demonstration of the catastrophic effect of minute traces of impurities. This led to the first reproducible measurements of hydrogen overvoltage through the use of adequate purification by adsorption (on platinum black) and by electrolysis. Similar careful studies of the charging curves of the platinum electrode were used to study the adsorption properties of this complex system for hydrogen and oxygen as well as electrolytes, and culminated in the proposal of the logarithmic adsorption isotherm as well as in the first use of the alternating current method for the measurement of the rate of electrode reactions. He later showed how this method may also be used for studying rates of adsorption.

During the second world war Frum-

kin was Director of the Colloido-Electrochemical Institute and, after the war, became for a time Director of the Institute of Physical Chemistry before, in 1958, a new Institute of Electrochemistry was formed with him as its Director, a post he held until his death. His own work broadened and extended in all directions in collaboration with the eminent coworkers he had attracted. This work was characterised by the intimate collaboration of experimentalists and theoreticians (notably V. G. Levich) which led to such developments as the rotating disc and ring-disc electrodes, the laws of photo-electric emission into solutions and the detailed understanding of the structure of electrified interfaces. His own interest in the last few years centred on the development and testing of the thermodynamic theory of interfaces across which charge may be freely transferred.

His encyclopaedic knowledge of all parts of electro- and surface-chemistry earned the admiration of everyone who knew him and his achievements were recognised by many honours (including the title of Hero of Socialist Labour, on his 70th birthday). He was also a man of broad general culture. He spoke English, French and German fluently and had a passionate interest in literature and the visual arts—he would travel far under difficult conditions to see an early church with fine frescoes. His other passion was mountaineering; naturally his activities were more restricted in recent years, yet as late as last September he organised a car trip from Alma Ata as high as possible into the Tien Shan range, and was delighted to find that he suffered no ill effects.

Roger Parsons

announcements

Appointments

Dr R. C. Smith as professor of Physical Electronics at the University of Southampton.

Drs J. B. Harborne and **D. M. Moore** as Professors of Botany at the University of Reading.

Professor Sir John Dacie has been nominated President-elect of the Royal Society of Medicine.

M. Hubert Curien has been appointed president of the Centre National d'Etudes Spatiales (CNES).

Meetings

August 2-6, **Cell Wall Biochemistry Related to Specificity in Host-Plant Pathogen Interactions**, Tromsø, Norway (Jan Raa, University of Tromsø, Tromsø, Norway).

August 17-19, **Water Relations in Membrane Transport in Plants and Animals**, Philadelphia (American

Physiological Society, c/o Dr Robert E. Forster, A-201 Richards Building G4, University of Pennsylvania, Philadelphia 19174).

August 23-27, **Congress of the International Primatological Society**, Cambridge (Dr D. J. Chivers, c/o Laundry Farm, Barton, Road, Cambridge, UK). September 7-9, **Arboricultural Conference**, Worplesdon, Guildford (P. H. Bridgeman, c/o Merrist Wood Agricultural College, Worplesdon, Guildford, Surrey GU3 3PE, UK).